The hyporheic zone as a refugium for benthic invertebrates in groundwater-dominated streams

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THE HYPORHEIC ZONE AS A REFUGIUM FOR BENTHIC INVERTEBRATES IN GROUNDWATER-DOMINATED STREAMS

BY

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A DOCTORAL THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
THE AWARD OF DOCTOR OF PHILOSOPHY OF LOUGHBOROUGH UNIVERSITY

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Abstract

A principal ecological role proposed for the hyporheic zone is as a refugium that promotes benthic invertebrate survival during adverse conditions in the surface stream. Whilst a growing body of work has examined use of this hyporheic refugium during hydrological extremes (spates, streambed drying), little research has considered variation in refugium use over prolonged periods including contrasting conditions of surface flow. In this thesis, benthic invertebrate use of the hyporheic refugium is considered at monthly intervals over a five-month period of variable surface flow, at nine sites in two groundwater-dominated streams, the River Lathkill (Derbyshire) and the River Glen (Lincolnshire). Conditions identified as potential triggers of refugium use included a flow recession and a high-magnitude spate on the Lathkill, and small spates and a decline in flow preceding localised streambed drying on the Glen. During flow recession, reductions in submerged habitat availability and concurrent increases in benthic population densities were dependent on channel morphology. An unusual paired benthic-hyporheic sampling strategy allowed the type of refugium use (active migration, passive inhabitation) to be inferred from changes in hyporheic abundance and the hyporheic proportion of the total population. Using this approach, evidence of active migrations into the hyporheic zone use was restricted to two instances: firstly, *Gammarus pulex* (Amphipoda: Crustacea) migrated in response to habitat contraction and increased benthic population densities; secondly, migrations of Simuliidae (Diptera) were associated with low-magnitude spates. Refugium use was site-specific, with refugial potential being highest at sites with downwelling water and coarse sediments. A conceptual model describing this spatial variability in the refugial capacity of the hyporheic zone is developed for low flow conditions. In some cases, hyporheic refugium use was apparently prevented by disturbance-related factors (rapid onset, high magnitude) regardless of the refugial potential of the sediments. The extension of the hyporheic zone's refugial role to include low flows highlights the need to explicitly protect the integrity of hydrologic exchange in river rehabilitation schemes. However, the limited capacity of the hyporheic refugium emphasizes the additional importance of maintaining habitat heterogeneity including multiple instream refugia.

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1. Introduction

1.1 Context

In recent decades, ecological research has placed an increasing emphasis on the importance of longitudinal, lateral and vertical linkages between river ecosystems and their surrounding environment (Vannote *et al*., 1980; Junk *et al*., 1989; Ward, 1989). Whilst research considering the vertical dimension has lagged behind longitudinal and lateral components, it is increasingly recognised that the subsurface sediments of the hyporheic zone are central to the ecological functioning of river ecosystems (Stanford and Ward, 1993; Boulton *et al*., 1998, 2010; Robertson and Wood, 2010). By connecting the surface stream with the underlying groundwater, hydrologic exchange through the hyporheic zone allows water, nutrients, organic matter and organisms to move between ecosystem components (Jones and Holmes, 1996; Brunke and Gosner, 1997).

A key ecological role proposed for the hyporheic zone is as a refugium that promotes persistence of benthic invertebrates (which typically inhabit the surface sediments) during adverse conditions in the surface stream (Orghidan, 1959, 2010; Williams and Hynes, 1974; Wood *et al*., 2010). To date, most research considering the hyporheic zone as a refugium has focussed on hydrological extremes, namely spates (Williams and Hynes, 1974; Giberson and Hall, 1988; Bruno *et al*., 2009) and streambed drying (Clifford, 1966; Cooling and Boulton, 1993; Del Rosario and Resh, 2000). Whilst some studies have demonstrated active use of the hyporheic refugium during both spate and drying events (Clinton *et al*., 1996; Marchant, 1995), evidence is equivocal and refugium use appears dependent on the fulfilment of environmental criteria (Smock *et al*., 1994; Olsen and Townsend, 2005). Little research has examined use of the hyporheic refugium during moderate hydrological conditions such as flow recession and low flows, and no previous study has linked declining discharge to increasing invertebrate abundance in the hyporheic zone (James *et al*., 2008; James and Suren, 2009; Stubbington *et al*., 2009a; Wood *et al*., 2010). This is despite the prediction that benthic invertebrates should migrate into the hyporheic zone during low flows if adverse biotic conditions (e.g. increased densities of predatory taxa) develop in a contracting submerged habitat area (Covich *et al*., 2003; James *et al*., 2008). In addition, little is known regarding how the refugial capacity of a particular area of hyporheic sediments may change during a temporal sequence of contrasting hydrological conditions.

Climate change scenarios for temperate regions such as the UK are rife with uncertainty (Wilby *et al*., 2008). However, many scenarios suggest that a range of hydrological conditions with the potential to stress invertebrate communities could become increasingly common, including high-magnitude floods (Christensen and Christensen, 2003), prolonged periods of low flow (Arnell, 2003; Fowler and Kilsby, 2007), and a greater occurrence of streambed drying (Shackley *et al*., 2001). A concurrent increase in water resource exploitation (including both surface and groundwater abstraction) is expected to exacerbate climatic effects on instream habitats and communities (Fowler *et al*., 2007). The hyporheic zone could play a crucial role in allowing persistence of invertebrate communities during any increase in the occurrence of adverse conditions. However, the ability of the hyporheic zone to function as an invertebrate refugium may be compromised by anthropogenic activities and hydrological conditions that clog interstices with fine sediments and reduce hydrologic exchange (Hancock, 2002; Boulton, 2007a).

The hyporheic zone is considered to be an ecotone (*sensu* Odum, 1971) between the surface stream above and the groundwater below (Orghidan, 1959; Williams *et al*., 2010). As such, invertebrate communities in surficial and hyporheic sediments show considerable overlap in species composition (Williams and Hynes, 1974; Davy-Bowker *et al*., 2006). However, the little research which has examined responses of both invertebrate assemblages to the same environmental stressor has shown that the benthic community response cannot be assumed to reflect that of the hyporheic fauna (Stubbington *et al*., 2009a; Wood *et al*., 2010). River restoration projects typically only consider the surface stream, which may limit potential benefits for the ecosystem as a whole (Boon, 1998; Bannister *et al*., 2005; Boulton *et al*., 2010). In particular, surface-focussed schemes may not improve hydrologic exchange, and may therefore have little effect on the hyporheic zone’s ability to support a diverse invertebrate community (Boulton, 2007a; Kasahara *et al*., 2009). Further research is required that simultaneously considers benthic and hyporheic community responses to changing environmental conditions. Such research is expected to demonstrate a need to explicitly consider the hyporheic zone in management strategies and restoration schemes aimed at maximising the ecological integrity of whole stream ecosystems (Boulton *et al*., 2010).

The research presented in this thesis has applied an unusual paired benthic-hyporheic approach to a field sampling programme, allowing concurrent examination of responses of both invertebrate communities to the same hydrological stimuli (Stubbington *et al*., 2009a; Wood *et al*., 2010). The findings of this research contribute to the growing literature highlighting the crucial ecological role played by the hyporheic zone. Such research should help to guide holistic management strategies and restoration initiatives aimed at maximising the ecological integrity of stream ecosystems in the face of increasing anthropogenic and climatic pressures.

1.2 Research aims and objectives

The overall aim of this research is to relate use of the hyporheic zone by benthic invertebrates to hydrological variability in the surface stream. Use of the general term ‘hydrological variability’ is intentional, due to the impossibility of predicting conditions that will be experienced over a predefined period. To counteract this inherent uncertainty, samples were collected at regular intervals over a prolonged (five month) period, thus increasing the probability of characterising use of the hyporheic zone during a range of contrasting hydrological conditions. In addition, sampling was conducted at multiple locations within two groundwater-dominated rivers, the hydrological characteristics of which further increased the probability of encountering conditions spanning the hydrological spectrum (i.e. spates, low flows and streambed drying; Chapter 3). An unusual combination of sampling techniques was adopted, to allow concurrent characterisation of benthic and hyporheic invertebrate communities, and to allow sample collection from the same locations on multiple occasions during a temporal sequence (Chapter 4). To complement the temporal emphasis of the research, spatial variability in key hydrological, sedimentological and chemical features of the habitat provided by the hyporheic zone was examined and related to observed refugium use (Chapters 5-7). Conducting identical sampling programmes in two comparable ecosystems facilitated identification of general patterns of community response, and guarded against the drawing of conclusions based on individual ecosystem characteristics.

This research explores relationships between the distribution of invertebrates on and within the streambed sediments, temporal variability in hydrological conditions, and spatial variability in the habitat provided by the bed sediments. Sampling was undertaken at a sub-reach scale, with replicate sampling points located in comparable habitat patches to minimise the confounding effects that could arise from high levels of heterogeneity typical of lotic ecosystems. Specifically, this research addresses the following aims and objectives:

Aim 1: Identify changes in hydrological conditions, hydrologically-mediated environmental conditions, and associated biotic variables with the potential to stress invertebrates in the benthic sediments.

Objectives (addressed by Chapters 5 and 6)

1. Examine temporal variability in surface hydrology for the period both during and preceding the study using continuous discharge data. A continuous record of discharge variability is required as context for explaining observed environmental and biological patterns, and will identify key instream disturbance events (spates, streambed drying) that may otherwise be overlooked by the necessarily coarse temporal resolution of the sampling strategy.
2. Set the hydrological conditions recorded during the study period in context using long-term data provided by external organisations. Use of such data allows observed conditions to be compared with those typically experienced by instream communities.

3. Identify the effects of discharge variability on the nature and extent of instream habitats by measuring site-specific variables and making on-site observations. Discharge-related changes in habitat availability and heterogeneity may have detrimental effects on benthic invertebrate communities.
4. Identify changes in ecologically-relevant physicochemical properties of water that arise as a result of hydrological variability. Variation in surface flow can have pronounced and sometimes detrimental impacts on water quality, which has the potential to stress instream fauna.
5. Infer hydrologically-mediated changes in the strength of biotic interactions within the benthic sediments from changes in the abundance of dominant taxa. Habitat contraction during flow recession may concentrate invertebrates into a smaller inhabitable space, with the consequent development of adverse biotic conditions for many taxa.
6. Characterise key features of disturbance events identified as potential invertebrate stressors, which may influence the benthic community response. Disturbance magnitude, duration, predictability and rate of onset are potentially important influences on community response.

Aim 2: Examine the response of the benthic invertebrate community to identified environmental and biotic stressors, with particular focus on the use of the hyporheic zone as a refugium.

Objectives (addressed by Chapters 5 and 6)

1. Investigate temporal change in benthic and hyporheic invertebrate community composition using community metrics and multivariate analysis. Community level analysis can facilitate assessment of the importance of spatial and temporal environmental variability in controlling assemblage composition.
2. Identify temporal changes in the abundance of common benthic taxa inhabiting the hyporheic sediments, and, in addition, analyse temporal changes in the proportion of the total (benthic + hyporheic) community residing within the hyporheic zone. Such analysis may facilitate inference of the nature of hyporheic zone inhabitation by benthic invertebrates.

3. Use multivariate analyses to examine relationships between environmental conditions and community metrics. Multiple environmental conditions may be identified as potential stressors of the benthic community, and multivariate analysis may identify the principal drivers of community change. Alternatively, weak environment-community relationships may highlight the greater importance of other (i.e. biotic) factors.
4. Investigate spatial variability in the suitability and use of the hyporheic zone as a refugium, with particular reference to hydrological characteristics (flow permanence; direction and strength of hydrologic exchange), relatively stable habitat parameters (sediment composition) and temporally variable environmental factors (water quality). These factors may influence use of the hyporheic zone refugium, or disturbance-related variables (e.g. magnitude, rate of onset, duration) may be of greater importance (also see Chapter 7).

Aim 3: Develop conceptual models relating spatial variability in habitat characteristics to use of the hyporheic zone by benthic invertebrates during hydrologically-mediated disturbance events affecting the surface sediments. It is intended that such models will highlight important characteristics of hyporheic sediments with high refugial potential, and as such will help guide holistic stream restoration programmes and inform future interdisciplinary research efforts (Chapters 7 and 8).

1.3 Structure of the thesis

The thesis structure and the corresponding development of the research are summarised in Figure 1.1. Chapter 1 has provided the broad context for the project, highlighted current research gaps and set out how the aims and objectives of the current project will address these gaps. Chapter 2 provides a more comprehensive overview of the research field, examining the importance of disturbance events in structuring instream communities and emphasizing the role of refugial habitats (in particular the hyporheic zone) in promoting community persistence during adverse conditions in the surface stream. Research gaps related to factors

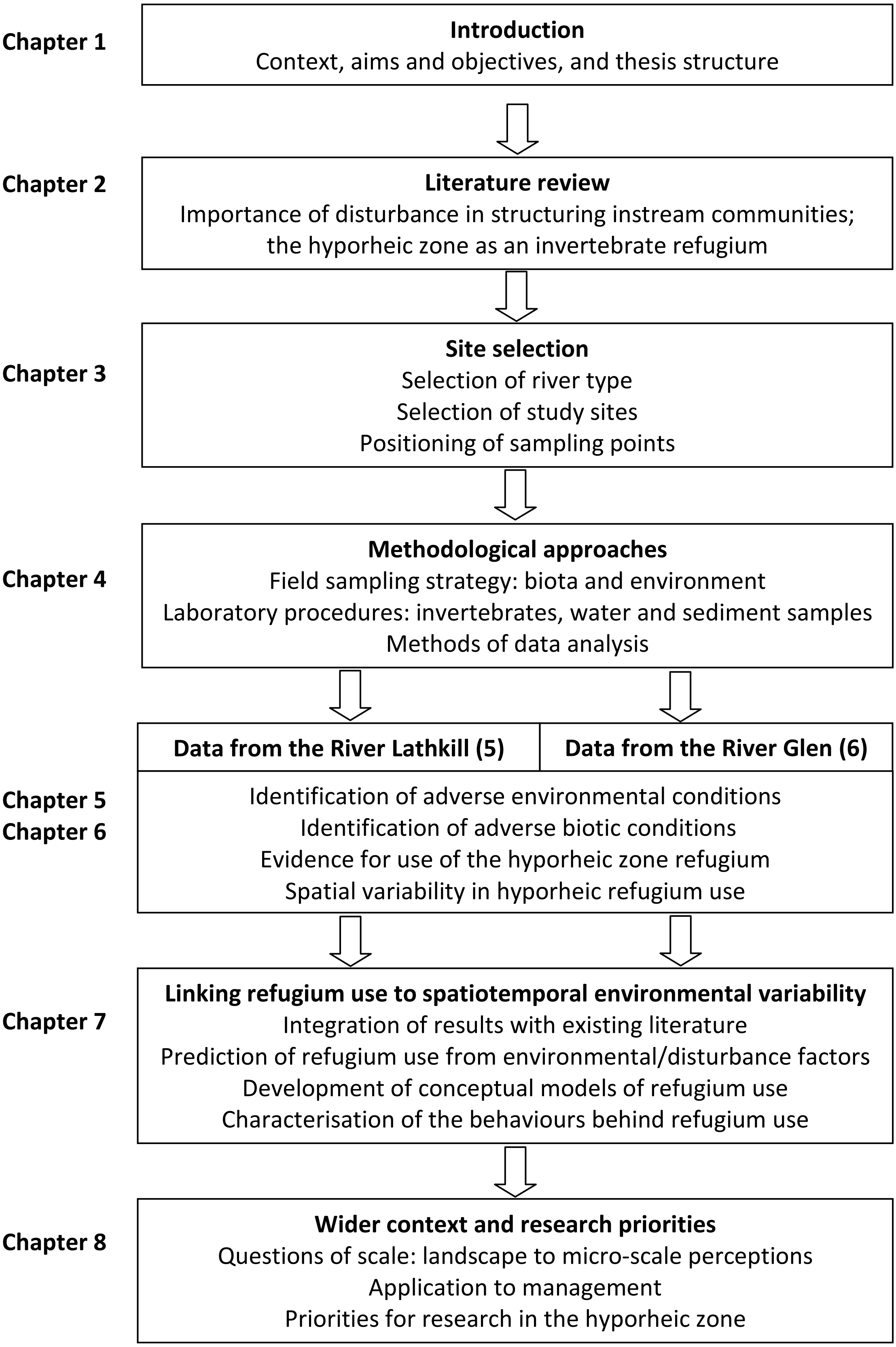


Figure 1.1. Schematic representation of the thesis structure.

controlling the use of the hyporheic zone as a refugium are addressed, thus emphasizing the need for the current research. In Chapter 3, the process of site selection is outlined, from the rationale governing choice of river type and locations of individual sites to the positioning of sampling points within each site. Chapter 4 describes the methodological approach adopted, including the field sampling programme, details of laboratory procedures, and methods of data analysis. Issues related to the collection of comparable invertebrate samples from benthic and, in particular, hyporheic habitats are discussed in Chapter 4 and rationale behind the selected sampling strategy is provided.

Organisation of the main research findings reflects the application of the same sampling programme to two comparable rivers, with the main results being presented first for the River Lathkill (Chapter 5) and subsequently for the River Glen (Chapter 6). For each river, the hydrological conditions experienced are outlined and set in long-term context, then temporal change in instream conditions is described and environmental stressors identified. Temporal change in the benthic community is examined, with the particular intention of identifying changes in abundance with the potential to increase biotic interactions. Hyporheic community composition is then examined, with a focus on spatiotemporal variability in the benthic component of the assemblage. Key results from the Lathkill and Glen are discussed in the context of existing literature in Chapters 5 and 6 respectively, and are subsequently compared in Chapter 7.

Conceptual models summarising spatial variability in the hyporheic zone refugium during key instream disturbances are also developed in Chapter 7. In addition, the possibility of predicting refugium use from environmental features of the hyporheic sediments and characteristics of disturbance events is explored. Results from both rivers are then used to develop a tool from which invertebrate behaviour can be characterised from changes in benthic abundance, hyporheic abundance and the hyporheic proportion of the total population. Chapter 8 highlights the key advances the project has made to the understanding of the hyporheic zone’s ecological importance, and emphasizes the importance of a holistic approach (which explicitly incorporates the vertical dimension) in river management and restoration schemes. Finally, Chapter 8 outlines the ongoing need for further interdisciplinary research within the hyporheic zone and encourages the exploitation of new technologies to address remaining research gaps.

2. Literature review: the hyporheic zone as an invertebrate refugium during instream disturbance

2.1 Introduction

This chapter provides a detailed review of existing literature in two related areas of freshwater invertebrate ecology: the effects of disturbance events including spates, low flows and streambed drying on instream communities, and the importance of adaptations in promoting survival during such events. Behavioural adaptations that enhance survival of benthic invertebrates are considered for each disturbance type and particular attention is paid to the use of the hyporheic zone as a refugial habitat. Research gaps are identified in each area, with use of the hyporheic zone refugium being identified as a particular area where knowledge remains incomplete.

2.2 The ecological importance of hydrological variability

That the flow regime is a central influence on instream habitats and communities is fundamental to research in lotic ecosystems (Statzner and Higler, 1986; Poff *et al*., 1997; Lancaster and Mole, 1999; Monk *et al*., 2007). Natural spatiotemporal hydrological variability is vital in maintaining the ecological integrity of lotic ecosystems (Bunn and Arthrington, 2002; Richter *et al*., 2003; Arthrington *et al*., 2006) and influences community composition in assemblages including riparian vegetation (Gilvear and Willby, 2006), diatoms (Growns and Growns, 2001), fish (Freeman *et al*., 2001) and invertebrates (Rader and Belish, 1999; Konrad *et al*., 2008). Several particular elements of the flow regime have been identified by previous research as being of ecological relevance, including: the *magnitude* of flow, which determines habitat availability; the *timing* of various conditions, including high and low flows, which determines if lifecycle requirements are met; the *frequency* with which certain conditions occur, which affects reproduction and mortality; the *duration* of certain conditions, which controls completion of particular life stages; and the *rate of change* in water conditions, which influences behavioural responses (Richter *et al*., 1996; Wood *et al*., 2001). In addition, intra- and inter-annual flow variability is important in maintaining ecosystem integrity, due to the positive correlation between flow variability and instream habitat heterogeneity (Jowett and Duncan, 1990; Bickerton, 1995; Poff *et al*., 1997).

2.2.1 Hydrological conditions as disturbance events

The flow regimes of most systems have distinct seasonal fluctuations, for example English rivers are usually characterised by peak flows during winter and early spring and low flows during summer (Haines *et al*., 1988; Bower and Hannah, 2002). This variability is vital in maintaining ecosystem integrity and fluctuations in flow can have pronounced effects on instream communities. At one extreme of the hydrological continuum, spates occur, and at the other extreme, streambed drying affects both naturally intermittent and usually perennial streams. Both conditions can cause significant changes in community structure (e.g. Extence, 1981; Fritz and Dodds, 2004), and in particular, the duration for which a substrate patch remains submerged is recognised as a crucial determinant of its ability to function as a habitat for aquatic biota (Frissell *et al*., 1986; Wright, 1992; Smith and Wood, 2002; Smith *et al*., 2003; Wood *et al*., 2005; Fenoglio *et al*., 2007).

The profound effects of certain hydrological conditions on instream habitats and communities have led to their consideration as disturbance events. Lake (2000), for example, considers a disturbance as occurring through the application of potentially damaging forces to a space inhabited by a population or community. However, the element of predictability is also recognised as important in defining whether a condition affecting a particular ecosystem should be considered a disturbance. Reflecting this, a widely cited definition is that of Resh *et al*. (1988, p.1) who consider a disturbance as 'any relatively discrete event in time that is characterised by a frequency, intensity and severity outside a predictable range that disrupts ecosystem, community or population structure'. This definition emphasizes that a disturbance can be considered as two sequential events: the disrupting force of the disturbance itself, and the resultant ecological response observed in instream communities (Glasby and Underwood, 1996; Lake, 2000; Lake 2003). These disturbances are regarded as a principal factor in structuring instream communities in lotic freshwater ecosystems (Robinson and Minshall, 1986; Resh *et al*., 1988; Poff, 1992).

2.3 Effects of hydrological disturbances on instream habitats

Regardless of their predictability and thus status as disturbance events, various hydrological conditions can have profound effects on habitat structure and function. Below, the contrasting effects of spate and drought conditions (including flow recession, low flows and streambed drying) are considered, with particular reference to disturbance features of relevance to invertebrate fauna.

2.3.1 Effects of spates on instream habitats

Spates occur in almost all natural lotic systems, although their magnitude, duration, frequency and predictability vary widely depending on climate and geology (Poff *et al*., 2006). These events are 'pulse' disturbances (*sensu* Lake, 2000) that occur suddenly and quickly reach maximum disturbance strength, and whilst these events are inherently unpredictable, spates of a given magnitude generally have a predictable return period (Poff and Ward, 1989). Despite their typically short duration (hours to days), spates are often of a sufficient magnitude to have severe impacts on the instream environment.

Essentially, spate flows involve a greater than average volume of water moving rapidly in a downstream direction, which creates high shear stress at the sediment surface (Death, 2008). Spate flows can result in entrainment of sediment, with fine sediment becoming suspended in the water column and deposited downstream, whilst larger clasts can also be transported shorter distances; the size of the particles displaced depends on the strength of shear stress (Petit, 1987). Through this bedload movement, spates are considered amongst the most important factors influencing the character of both surficial and hyporheic streambed sediments. Scour of sediment from some locations (Matthaei *et al*., 1999) and deposition of material in other areas (Olsen *et al*., 2010) contributes to the heterogeneity of instream habitat patches (Olsen and Townsend, 2005) as well as influencing large-scale adjustments in channel morphology (Gilvear, 2004). This reworking of the bed sediments can have various secondary effects including a change in the direction and strength of hydrologic exchange through the bed sediments (i.e. upwelling or downwelling water; Dole-Olivier and Marmonier, 1992a; Wondzell and Swanson, 1999) and the burial or removal of organic matter (Naegeli *et al*., 1995; Lytle, 2000). In addition, fast-flowing flood waters can damage or remove habitat provided by bryophytes, macrophytes and riparian vegetation (Suren and Duncan, 1999; Hancock, 2006; McKenzie-Smith *et al*., 2006) and can scour epilithic biofilms from surfaces (Scrimgeour *et al*., 1988). The physicochemical properties of water can also be affected due to an alteration in groundwater influence, reduced groundwater residence times (Brunke and Gosner, 1997), dilution effects or increased inputs from the surrounding catchment (Reader and Demsey, 1989).

2.3.2 Definition and features of hydrological droughts

Considering the other extreme of the hydrological spectrum, the term ‘drought’ has been inconsistently defined (Whilite and Glantz, 1985; Boulton, 2003) and is used in a wide range of contexts, from meteorological droughts to socio-economic droughts (Gonzalez and Valdes, 2005). In hydrological terms, droughts are defined as a deficit in surface water or groundwater (in comparison with the long-term average (LTA); Fleig *et al*., 2006), which is observed instream as flow recession, low flows and partial or complete streambed drying. The ultimate cause of a hydrological drought is a meteorological drought (a deficit in precipitation in comparison with the LTA; Fleig *et al*., 2006). Humphries and Baldwin (2003, p. 1142) describe drought from the perspective of river ecology as ‘an unpredictable low flow period, which is unusual in its duration, extent, severity or intensity’. It may, however, be more appropriate to classify ecological droughts into two distinct groups according to their predictability (Lake, 2003). The first, seasonal droughts, are commonplace in regions with distinct seasonality, for example the warm, dry summers of the Pacific Northwest of the USA (Dieterich and Anderson, 2000) and the Mediterranean (Fonnesu *et al*., 2005), resulting in streams with predictably intermittent flow regimes. Despite receiving little attention in the literature, many rivers in temperate regions also experience seasonal summer drying, particularly those flowing over free-draining lithologies such as karst limestone (Meyer and Meyer, 2001; Stubbington *et al.*, 2009b). The second type of drought, supra-seasonal drought, describes events which are unpredictable in both their timing and duration (Lake, 2003), and which may span many seasons (Boulton and Lake, 2008). Supra-seasonal droughts can affect streams in any climate or region, for example English lowlands (Extence, 1981; Wood and Armitage, 2004) and Caribbean rainforests (Covich *et al.*, 2003).

In contrast to spates, droughts are ‘creeping’ events with no obvious onset (Tannehill, 1947), and their recognition as disturbances may be delayed until their magnitude exceeds a certain threshold (Humphries and Baldwin, 2003; Boulton and Lake, 2008). As such, droughts may act either act as ‘ramp’ disturbances (*sensu* Lake, 2000) which gradually increase intensity over a prolonged period, or as ‘press’ disturbances (*sensu* Lake, 2000), which are characterised by an abrupt onset followed by a plateau stage at the level of maximum disturbance. Typically, supra-seasonal droughts in largely perennial systems are ramp events in which flow gradually declines over a prolonged period, whilst seasonal droughts in intermittent streams can be considered as press disturbances if the loss of water from the surface stream is taken as the level of maximum disturbance (Lake, 2000).

Hydrological drought resulting from a deficit in precipitation may be exacerbated in lotic environments by both natural and anthropogenic activity. Natural phenomena include sinkholes and other features of karst bedrock, through which water may be continuously or intermittently lost, resulting in localised streambed drying during drought conditions (Hindley, 1965; Meyer and Meyer, 2000). Anthropogenic activities that increase pressure on water resources centre on abstractions for domestic and industrial uses, with demand tending to peak when flows are naturally at their lowest (Dewson *et al.*, 2007b). Bickerton (1995), for example, noted that groundwater levels in the River Glen catchment in eastern England have been declining since 1940, whilst small perennial streams in New Zealand are also reported as being under increasing anthropogenic pressure (Dewson *et al.*, 2007b). There is some evidence to suggest that global climate change already has increased the occurrence of hydrological drought in the UK (Morris and Marsh, 1985;

Hannaford and Marsh, 2006), and it is predicted that the frequency and intensity of both single-season and supra-seasonal events will continue to increase (Lake, 2003; Defra, 2006), further increasing pressure on water resources. Compounding these proposed changes, past human endeavour can have ongoing hydrological effects, for example drainage levels constructed to aid lead mining activity in past centuries continue to remove water from surface channels in parts of Derbyshire's Peak District (James, 1997; English Nature, 2004).

It is debatable whether predictable drought events should be considered as disturbances at all, and the instream conditions that characterise drought can also occur during periods of above-average discharge. Nonetheless, the influence of these conditions on instream habitats and communities is indisputable, with flow recession, low flows, and streambed drying all having marked effects.

2.3.3 Effects of flow recession and low flows on instream habitats

Flow recession and low flows are hydrological components of wider drought disturbances (Boulton, 2003; Lake, 2003) and can also characterise summer flow regimes in years with average or above-average precipitation. Despite being less dramatic conditions than spates, reductions in flow can also have marked effects on instream habitats (Dewson *et al.*, 2007a).

As streamflow declines during flow recession, a typical sequence of events unfolds (Figure 2.1(i)), starting with the isolation of the channel from the surrounding landscape. Lateral connectivity with the floodplain has been recognised as facilitating movement of organisms, nutrients and organic matter between the river and its catchment (Junk *et al.*, 1989; Ward, 1989; Thorp *et al.*, 2006), but this is severely restricted as discharge declines. Soon after, links are cut with the habitat and allochthonous food resources provided by the riparian zone (Wright and Berrie, 1987; Tabachi *et al.*, 1998; Harrison, 2000; Figure 2.2(ii)). Next, further reductions in water depth occur, although concurrent declines in wetted width (which determine the extent of habitat contraction) are dependent on channel morphology (Cowx *et al.*, 1984; Dewson *et al.*, 2003; Gordon *et al.*, 2004). Wright and Symes (1999), for example, found that there was no decline in the channel width of an English chalk stream despite a major reduction in discharge during a drought. At the same time, lentic and slow-flowing habitats may become more prevalent (Wright *et al*., 2002), but overall the hydraulic heterogeneity of flow decreases and fast-flowing habitats may be lost (McIntosh *et al*., 2002; Lake, 2003). A decline in current velocity also reduces the longitudinal transport of fine sediment and particulate organic matter, resulting in their deposition and on the substrate surface (Wright and Berrie, 1987; Wright, 1992; Wood and Petts, 1999; McKenzie-Smith *et al*., 2006; Figure 2.2(ii)).

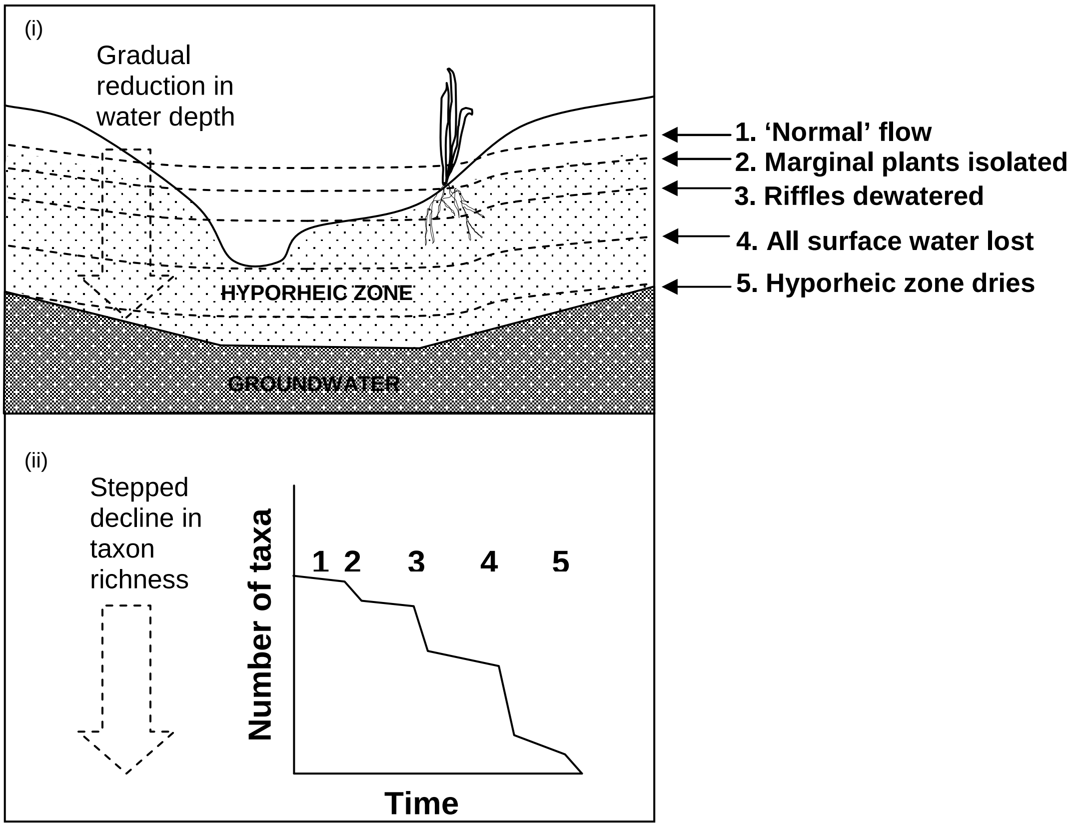


Figure 2.1: (i) Stream cross-section, indicating critical stages for invertebrate biota during a gradual decline in discharge; (ii) 'Stepped' changes in taxon richness corresponding to critical stages (adapted from Stubbington *et al*., 2009a; Boulton, 2003).

As the drought continues, contraction of submerged habitat affects many streams, with surface water being lost first from shallow reaches such as riffles and margins. If the drought persists surface flow may cease altogether, at which point the stream channel becomes a series of disconnected pools (Boulton and Lake, 1990; Fritz and Dodds, 2004; Bonada *et al*., 2006; Figure 2.2(iii)); at this point, habitat availability may also decline in the hyporheic zone (Figure 2.2(iv)). The formation and duration of pools is dictated by the extent to which the groundwater table declines and by

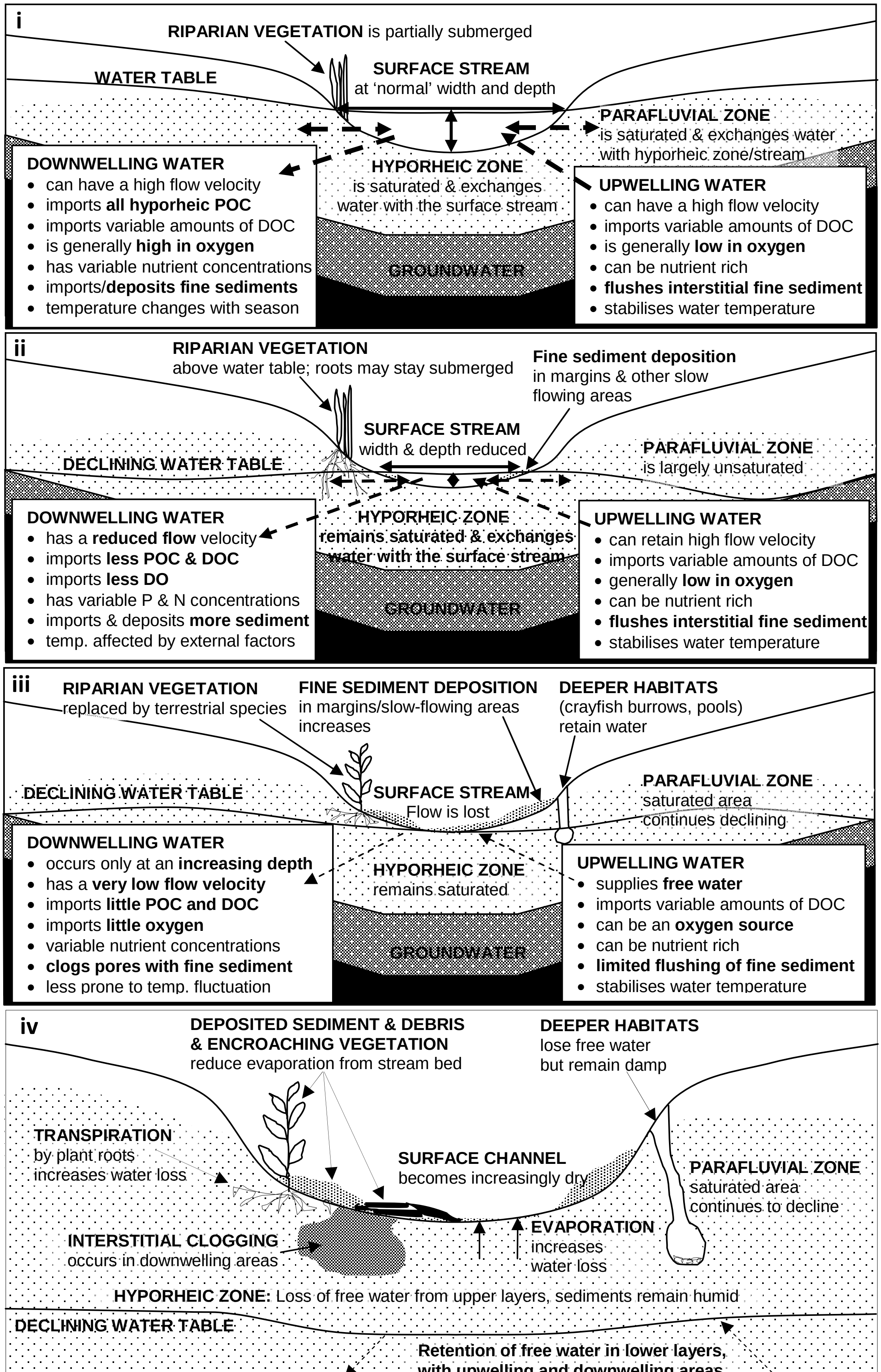


Figure 2.2: Factors affecting the integrity of the hyporheic zone habitat during flow recession: i) 'normal' flow; ii) low flow; iii) loss of surface flow; iv) loss of saturated hyporheic habitat. DOC = dissolved organic carbon; POC = particulate OC. (Adapted from Stubbington *et al.*, 2009a).

the permeability of the bed sediments (Williams and Hynes, 1977). Many pools are short-lived, but some may persist in deeper sections with a relatively impermeable substratum. These pools retain some connectivity only if flow occurs in peripheral subsystems such as the hyporheic zone (e.g. Wood, 1998).

Throughout a flow recession, external stimuli such as solar radiation and groundwater have an increasing influence on the physicochemistry of the remaining body of water (Dewson *et al*., 2007a). Since solar radiation and air temperature are frequently high when flows are at their lowest, water temperature typically increases (Cowx *et al*., 1984; Hakala and Hartman, 2004), particularly in remnant pools (Boulton and Lake, 1990). Some studies, however, have recorded a decrease in stream water temperature during low flows, this being attributed to a greater influence of groundwater inputs (Grant, 1977; Mosley, 1983). Any increase in temperature can also contribute, together with a reduction in physical turbulence and increased groundwater influence, to a decline in dissolved oxygen concentrations (Boulton and Lake, 1990; Elliot, 2000). Both increased groundwater influence and reduced dilution can result in an increase in electrical conductivity (Chessman and Robinson, 1987; Caruso, 2002; Lind *et al*., 2006).

A reduction in flow has various effects on the biogeochemical processes that control ecosystem functioning, including the transport and transformation of nutrients and energy (Baldwin and Mitchell, 2000). The mechanisms supplying nutrients and energy to a given location may change, and concentrations of dissolved organic carbon, nitrogen and phosphorus may be altered (Baldwin and Mitchell, 2000; Dahm *et al*., 2003). Due to the loss of connectivity with the adjacent floodplain, the importance of upwelling groundwater as a source of nutrients can increase (e.g. Boulton and Stanley, 1995), particular in unenriched rivers (Suren *et al*., 2003a). Concentrations of nutrients may increase or decrease. Ladle and Bass (1981), for example, ascribed an increase in potassium and phosphate concentrations prior to stream drying to reduced dilution by inputs from the surrounding catchment. In contrast, Caruso (2002) attributed a decline in nitrogen and phosphorus concentrations during severe low flows in New Zealand to reduced inputs from rainfall, run-off and diffuse sources, whilst Dahm *et al*. (2003) ascribed similar declines to the increased influence of low nutrient groundwater inputs.

Reduced discharge in the surface stream can also affect processes within the subsurface hyporheic zone, even when surface water remains connected. Whilst the strength of hydrologic exchange is likely to correspond to any reduction in surface velocities (Hancock, 2002; Figure 2.2), some studies have found hyporheic exchange pathways to become more diverse (Marmonier and Creuzé des Châtelliers, 1991). In addition, downwelling surface water provides subsurface sediments with a regular influx of organic matter during low flows (Marmonier and Creuzé des Châtelliers, 1991). In addition, fine sediment deposited on the streambed can be carried into the hyporheic zone and can clog interstitial spaces (the process of colmation), which compromises hydrologic exchange and impacts upon the transport of water, nutrients, organic matter and organisms (Milan and Petts, 1998; Brunke, 1999; Hancock, 2002; Figure 2.2(ii-iv)).

2.3.4 Effects of streambed drying on instream habitats

Partial streambed drying (i.e. habitat contraction) typically occurs during the sequence of events that accompany a decline in discharge (Figure 2.1). In addition, following the loss of connected, flowing water and the formation of pools, the most critical change to the integrity of instream habitat may occur: complete loss of surface water (Boulton, 2003). As the medium which biotic communities inhabit, free water is the most crucial element of freshwater ecosystems and its loss is of immense ecological relevance. Following the loss of surface water, subsurface water may be retained in the hyporheic zone, particularly in areas of upwelling groundwater. However, the loss of surface flow also has marked effects on the physical environment within the hyporheic zone. First and foremost, there may be a significant alteration of its spatial configuration (e.g. Clinton *et al*., 1996), particularly as disturbance duration and magnitude increase. In addition, lack of flow coupled with an increased residence time of organic materials can cause hypoxia to occur, resulting in changes to biogeochemical processes (Fisher *et al*., 1998). Smock *et al*.

(1994), for example, found that anoxic conditions occurred just 3-5cm below the sediment surface during a drought in a temporary headwater stream. Similarly, Stanley *et al*. (1994) reported regular occurrence of anoxia in the hyporheic sediments following drying of a Sonoran Desert stream.

2.4 Effects of disturbance on benthic invertebrate communities

Perturbation in lotic ecosystems comprises two sequential components: the physical disturbance and the biotic response to this disturbance (Lake, 2000). The hydrological conditions outlined above (spates, low flows and streambed drying) may or may not be defined as a disturbance depending on predictability, but can nonetheless have profound effects on instream communities including macroinvertebrate fauna. Effects can be exerted through primary impacts such as high shear stress during spates and loss of surface water during droughts, and through secondary effects such as reduced water quality and changing resource availability during low flows.

2.4.1 Effects of spates on benthic invertebrates

Studies reporting the effects of spates on invertebrates inhabiting benthic sediments almost invariably record declines in abundance (Table 2.1). Reductions in the total abundance of invertebrates are often substantial, for example Fritz and Dodds (2004) found benthic population densities to be reduced by >99 % after a >50 year recurrence interval flood; Olsen and Townsend (2005) noted reductions of 89 % following an event with a 1.5 year estimated return period; and Mesa (2010) observed declines of 61 % after annual spates during the monsoon season in an Andean stream. Whilst a large proportion of the whole community may be removed during a spate, effects of high flows are nonetheless taxon-specific (Holomuzki and Biggs, 2000) and through disproportionate displacement of competitive and dominant taxa, spates can increase community diversity (Fisher, 1983; Power *et al*., 1988; Olsen and Townsend, 2005). Such increases in diversity may be precluded if the number of taxa present is also significantly reduced (e.g. Death and Winterbourn, 1995; Effenberger *et al*., 2008). However, unlike the consistent reports of decreases in abundance, some studies have recorded no difference in taxon richness pre-and post-flood (Reice, 1985; Rempel *et al*., 1999; Mesa, 2010), apparently due to the persistence of adapted taxa at low abundance.

During events that initiate bedload movement, the mechanisms by which invertebrates are displaced are clear, with mobilised sediment particles undoubtedly capable of dislodging benthic organisms and increasing involuntary entrance into flow (i.e. catastrophic drift; Brittain and Eikeland, 1998). In addition, it is suggested that mobile sediment particles may kill or injure invertebrates directly (Death, 2008). However, during smaller spates which do not rework the substrate, reasons for reductions in abundance are less clear (Bond and Downes, 2003). Some research has indicated that increased shear stress alone is sufficient to force invertebrates into the water column (Lancaster and Hildrew, 1993a; Bond and Downes, 2003), whilst other studies suggest abrasion by suspended fine sediment as an important factor (Downes *et al*., 1998). In addition to involuntary displacement, spates may cause behavioural (i.e. voluntary) drift to increase, with a change in current velocity recognised as an important a drift initiator as the actual velocities reached (Anderson and Lehmkuhl, 1968; Irvine, 1985; Perry and Perry, 1986).

In contrast to the typical patterns of declines in abundance and richness, a few studies taking a more holistic view of instream habitat use during spates have demonstrated that effects may not be as pronounced as first appears. Rempel *et al*. (1999), for example, found that invertebrate abundance did decline in some habitat patches disturbed by annual flooding in a large unregulated river, but that this was due to lateral migrations into slower-flowing marginal areas. These field observations are supported by experimental evidence demonstrating that invertebrates alter their movement behaviour to reduce exposure to high flow velocities and high turbulence (Rice *et al*., 2007). Such results highlight the importance of refugial habitats in promoting invertebrate persistence during adverse conditions in the surface channel.

2.4.2 Community response to drought disturbances

Compared to spates, the effects of drought on instream communities are poorly understood (Boulton and Lake, 2008), reflecting the difficulties in studying unpredictable events with no distinct onset. Droughts also have far more variable impacts on invertebrate community composition compared to spates (Table 2.1-2.3), due to disturbance parameters including the magnitude and duration of the decline in discharge, which together with channel morphology, determine the changes experienced in instream habitats (i.e. isolation of riparian zone, habitat contraction, formation of pools and/or complete streambed drying; section 2.3.3; Figure 2.1(i)). In addition, whilst the physical impacts of drought have been conceptualised as 'ramp' or 'press' disturbances (section 2.3.2), invertebrates may exhibit different types of response. When instream conditions are changing only gradually, communities may also exhibit a 'ramp' response. However, a 'stepped' model of community response may be more appropriate to describe rapid changes in community composition when a 'critical threshold' (e.g. the complete loss of surface water) is transcended (Boulton, 2003; Figure 2.1(ii)).

Table 2.1: Effects of spates on benthic invertebrate abundance, richness and diversity

SITE DESCRIPTION	SPATE MAGNITUDE	EFFECTS ON BENTHIC INVERTEBRATES	REFERENCE
Fourth-order gravel- to cobble-bed stream, N Carolina, USA	Experimental bedload movement	21-95 % reduction in abundance of each taxon. Taxon richness and community diversity remained constant	Reice, 1985
Third-order coastal stream, north California, USA	Highest discharge during 7 year study	Overall densities decreased but Chironomidae increased; concurrent decline in taxon richness and community diversity.	McElravy *et al*. 1989
Flood-prone river, New Zealand	Two high-magnitude, bed-moving floods	Severe reduction in invertebrate densities	Scrimgeour and Winterbourn, 1989
Bypassed section of Rhône River, France	High magnitude spate, mobile substratum	Benthic invertebrate abundance reduced in hyporheic sediments due to entrance into drift	Dole-Olivier *et al*., 1997
Pre-alpine river, Switzerland	High magnitude event (5 yr return interval)	Total invertebrate abundance reduced by 90 %; significant reduction in taxon richness (22 to 15 taxa)	Matthaei *et al*., 1997
Large unregulated gravel-bed river, Canada	Long-duration (two-month) seasonal flood	Invertebrates response to flood onset by moving to marginal, slow-flowing habitats; taxon richness not affected	Rempel *et al*., 1999
Small upland streams, Australia	Repeated small experimental spates	Significant decrease in invertebrate abundance and taxon richness due to observed entry into drift	Bond and Downes, 2003
Intermittent Prairie stream, Kansas, USA	High magnitude spate (>50 year return period)	Invertebrate richness reduced by >97 % and densities reduced by >99 %	Fritz and Dodds, 2004
Flow-regulated river, Switzerland	Series of experimental spates of varying magnitude	Macroinvertebrate densities reduced by 14-92 % depending on flood magnitude	Robinson *et al*., 2004
Fourth-order gravel-bed stream, New Zealand	Double-peak bed-moving spate and flood	Benthic and hyporheic invertebrate abundance and taxon richness lower post-flood than pre-flood; increase in community evenness	Olsen and Townsend, 2005
Two small streams, English Lake District	High magnitude spate	Species-specific reductions in abundance of four Elmidae beetles.	Elliott, 2006
Flood-prone stream, Germany	Bed-moving experimental flood	Decreased in invertebrate densities and taxonomic richness in unstable patches	Effenberger *et al*., 2008
Neotropical Andean stream, Argentina	High magnitude seasonal spate	Abundance reduced by 61%; taxon richness not affected; community evenness increased	Mesa, 2010

Table 2.2: Effects of low flows on benthic invertebrate abundance, richness and diversity

SITE DESCRIPTION	FLOW REDUCTION	EFFECTS ON BENTHIC INVERTEBRATES	REFERENCE
Prairie river, Montana, USA	Flow reduced by 60-87 % for 6 weeks	Increased population densities due to habitat contraction	Gore, 1977
Lowland river, England	Reduced flow at three sites. Flow cessation at one site.	General increase in population densities due to habitat contraction; some taxa (*Gammarus pulex*, cased caddisfly larvae) eliminated.	Extence, 1981
Small upland stream, Wales	Flow reduced by 60 % for 3 months	Reduced abundance of invertebrates during drought, subsequent change in community structure	Cowx *et al*., 1984
Lowland river, Australia	Record low flows, lack of waster water dilution	Little change to taxonomic richness or faunal composition of macroinvertebrate community.	Chessman & Robinson, 1987
Lowland chalk stream, England	Reduced flow during summer 1976 drought	Reduced invertebrate abundance and diversity but taxon richness unaffected. A few taxa increased in abundance e.g. chironomids.	Wright & Berrie, 1987; Wright & Symes, 1999
Limestone river, England	Multiple low flow years between 1976-1994	Rheophilic species (e.g. Simuliidae, Rhyacophilidae) occur at reduced density due to habitat loss	Bickerton, 1995
Regulated Mediterranean-climate river, France	Reduced residual flow, reduced habitat diversity	Reduced taxon richness compared with non-regulated reaches	Cazaubon and Giudicelli, 1999
Headwater streams, Rocky Mountains, USA	Mild to severe reduction in flow below diversion	Mild flow alterations have few effects; severe reductions result in significant reductions in abundance, taxon richness and diversity	Rader and Belish, 1999
Mountain stream, Hawaii	Reduced flow due to diversion of >90 % of flow	Reduced abundance and taxon richness below diversions. Loss of decapod and amphipod crustaceans	McIntosh *et al*., 2002
Four streams, New Zealand	Abstraction-related flow reductions of 22-81 %	Increased invertebrate abundance due to altered habitat suitability and food resources; taxon richness unchanged.	Dewson *et al*., 2003
Nutrient enriched stream, New Zealand	Summer flow recession reduces discharge by 85 %	Increased invertebrate abundance in nutrient enriched streams, due to increased algal production	Suren *et al*., 2003a
Lowland chalk stream, England	Two supra-seasonal droughts during 8 yr study	Drought years characterised by low invertebrate abundance and high community diversity	Wood and Armitage, 2004
Gravel-bed river, New Zealand	Several low flow periods	Densities of most invertebrate taxa remained unchanged, 4 taxa declined after 9 months of low flow due to loss of habitat	Suren & Jowett, 2006
Intermittent Mediterranean-climate river, Australia	Reduced flow only, due to regulation.	Little detectable response.	Lind *et al*., 2006
Small streams, New Zealand	Experimental flow reductions of >89 %	Some increase in invertebrate abundance linked to habitat contraction; no change in richness or diversity	Dewson *et al*., 2007b

Table 2.3: Effects of streambed drying on benthic invertebrate abundance, richness and diversity in intermittent and (usually) perennial streams

SITE DESCRIPTION	EXTENT OF DRYING	EFFECTS ON BENTHIC INVERTEBRATES	REFERENCE
Small mountain stream, Wales (usually perennial)	Drying of the streambed	Few invertebrate taxa survive in an active form	Hynes, 1958
Small stream, Denmark (usually perennial)	Streambed dry for 2-3 months	Elimination of *Gammarus pulex* and its replacement by *Asellus aquaticus* (following resumption of flow)	Iversen *et al*., 1978
Small chalk stream, England (usually perennial)	Streambed dry for 4 month	Species specific responses, including increases, decreases and no change in abundance (following resumption of flow)	Ladle & Bass, 1981
Intermittent mountain stream, Poland	Streambed dry for 6 months	Elimination of taxa during dry period. Colonisation by specialist temporary stream taxa on re-wetting, then their replacement.	Kownacki, 1985
Intermittent chalk stream, England	Streambed remained dry in 1976	Severe impact on macroinvertebrate abundance and diversity	Wright & Berrie, 1987
Intermittent sand-bottomed stream, South Carolina, USA	Streambed dry for 6 months	Loss of all active aquatic individuals.	Smock *et al*., 1994
Sycamore Creek, Sonoran Desert stream	Cessation of flow	Severe invertebrate mortality after water loss. Community composition changes at hydrologically isolated sites.	Stanley *et al*., 1994
Intermittent karst stream, Germany	Dry phase of 1-7 months	Macroinvertebrate species richness and abundance decreases with increasing length of the dry phase (following resumption of flow)	Meyer & Meyer, 2000
Rainforest stream, Puerto Rica (usually perennial)	Drying of headwater riffles, isolated pools remain	Concentration of large shrimp taxa in pools, decreased reproductive activity in these species.	Covich *et al*., 2003
Intermittent Prairie stream, Kansas, USA	Streambed dry for 9 months	Severe reductions in invertebrate species richness and density.	Fritz and Dodds, 2004
Lowland limestone river, England (usually perennial sites)	Desiccation of two reaches, on two occasions	Severely reduced invertebrate community abundance and *Gammarus pulex* abundance, increase in Shannon Wiener diversity	Wood & Armitage, 2004
Intermittent Mediterranean climate river, Australia	Drying out to pools, causing an increase in salinity	Changes to macroinvertebrate assemblage composition, elimination of taxa that cannot tolerate salinity increase	Lind *et al*., 2006

2.4.3 Effects of low flows and streambed drying on benthic invertebrates

The terms ‘flow recession’ and ‘low flow’ are discussed with reference to the sequence of events (described in section 2.3.3) starting with the loss of connectivity with the floodplain and riparian zones, and culminating in some degree of habitat contraction. These conditions are associated with reductions in habitat availability, changes in current velocity (and therefore, potentially, habitat heterogeneity), reductions in water quality and increased deposits of fine material, all of which have the potential to alter invertebrate community composition. Progression to chains of isolated pools was not of primary relevance in the current investigation, whilst effects of streambed drying are considered briefly.

Changes in invertebrate abundance and taxon richness

If a decline is discharge isolates the riparian zone from the main channel, this removes the habitat required by the diverse range of aquatic taxa that live, feed, pupate and/or emerge in this lateral habitat (Ormerod *et al*., 1987; Harrison, 2000); such losses can reduce taxonomic richness of the total ecosystem. Low flow conditions can also reduce the abundance of submerged macrophytes, affecting a different group of invertebrates that rely on this vegetation for habitat and food resources, and thus further reducing taxonomic richness. Submerged macrophytes of the genus *Ranunculus*, for example, can support a high abundance of macroinvertebrates (Wright, 1992; Armitage and Cannan, 2000), but decline during periods of low flow (Ladle and Bass, 1981; Wright and Berrie, 1987; Bickerton, 1995), which has been attributed to a combination of reduced stream width and increased deposition of fine sediments. As a result, taxa such as Simuliidae (Diptera), which filter-feed whilst attached to macrophytes such as *Ranunculus*, may decline in abundance (Bickerton, 1995).

The absence of scouring flows during flow recession also encourages the development of an abundant periphytic community (Dewson *et al*., 2007b), particularly in nutrient-enriched streams where high-biomass algal mats can develop (Suren *et al*., 2003a); this alters the range of instream habitats and food resources available to resident macroinvertebrates, resulting in species-specific increases or decreases in population densities. Extence (1981), for example, found that reduced flow and increased algal production in an English lowland river resulted in greater densities of herbivores, whilst increases in detritivore taxa occurred due to organic matter deposition and filter feeders benefitted from increased hydraulic stability. Similarly, Wright and Berrie (1987) attributed a greater occurrence of chironomid larvae during a low flow year to the increased food resources supplied by algae and the increased habitat provided by deposited silt. Suren *et al*. (2003a) recorded an increase in total invertebrate densities only in nutrient-enriched streams that had experienced a rise in the abundance of filamentous green algae, whilst invertebrate densities remained stable in unenriched streams.

Other taxa are also vulnerable to population declines as flow recession progresses, with rheophiles (which prefer fast-flowing, well-oxygenated waters) amongst the first to be affected. Bickerton (1995), for example, recorded reduced densities of rheophilic Rhyacophilidae (Trichoptera) and Simuliidae larvae during a low flow period in an English chalk stream. Similarly, Wright and Berrie (1987) found both Simuliidae and Baetidae (Ephemeroptera) abundance to be reduced in an English chalk stream during a period of reduced flow. These taxon-specific declines often result in an overall decrease in invertebrate abundance, which is typically attributed to a combination of factors including decreased habitat availability, changing biotic interactions (e.g. increased predation and competition), and low quantity and quality of food resources (Cowx *et al*., 1984; Rader and Belish, 1999; McIntosh *et al*., 2002; Wood and Armitage, 2004; Dewson *et al*, 2007a). In addition, any alteration in taxonomic richness is invariably a decline (Cazaubon and Giudicelli, 1999; McIntosh *et al*., 2002), which can be attributed reduced habitat heterogeneity; such declines are therefore more pronounced in heterogeneous stream reaches, whilst areas with uniform flow may maintain their limited range of habitats (Dewson *et al*., 2007a).

Increases in invertebrate abundance and biotic interactions

Many studies have recorded increases in overall invertebrate densities as flow declines, which may either reflect a numerically stable community being concentrated into a reduced submerged area (Gore, 1977; Extence, 1981; Wright and Berrie, 1987; Fritz and Dodds, 2004) or, as described above, increased food resources for specific taxa (Extence, 1981; Wright and Symes, 1999; Dewson *et al*., 2003). Regardless of cause, where population densities rise, this can alter the strength and direction of biotic interactions such as predation, cannibalism and competition (Dewson *et al*., 2007a). Such impacts are most pronounced following the loss of connected surface flow and formation of isolated pools, since lateral and longitudinal escape routes are unavailable to most prey taxa (Extence, 1981; Covich *et al*., 2003). Low flows may also increase biotic interactions where flow remains connected by removing high-velocity predation-refugia for prey taxa. Simuliidae larvae, for example, typically select fast-flowing habitats despite a reduction in feeding efficiency, in order to reduce impacts of predatory stoneflies (Malmqvist and Sackmann, 1996). Where such favoured habitats do remain but are reduced in extent, competition for space and resources may become fierce (Lake, 2003; Dewson *et al*. 2007a), although this has yet to be documented.

Streambed drying

The presence of free water is clearly essential to the survival of freshwater biota, and following the complete loss of surface water, impacts on invertebrate community that remains in the dry surficial sediments are invariably severe (Table 2.3). Major reductions are always recorded in both abundance and taxonomic richness (Hynes, 1958; Wright and Berrie, 1987; Stanley *et al*., 1994; Fritz and Dodds, 2004) and often all aquatic individuals are lost (Kownacki, 1985; Smock *et al*., 1994). As such, streambed drying can potentially have the most detrimental impacts on benthic invertebrates of the hydrological conditions under consideration.

2.4.4 Effects of streambed drying on hyporheic invertebrates

Droughts of sufficient magnitude not only affect surface water habitats, but also the physical characteristics and water chemistry of the hyporheic zone, particularly if surface water is lost. In turn, this may impact upon the hyporheic zone's resident biota, i.e. the hyporheos (Williams and Hynes, 1974). Following surface drying, if the hyporheic zone retains free water then it has the potential to act as a refugium for benthic invertebrates (see section 2.7) However, if the water table declines sufficiently, the shallow layers of the hyporheic zone can also become dry, resulting in 'subsurface drought' (Boulton, 2003). Such drying can result in a spatial redistribution of the hyporheos, potentially including reduced faunal abundance in the shallow hyporheic sediments and a concurrent increase in abundance in the deeper layers, reflecting a migration that follows the receding water level (Griffith and Perry, 1993; Clinton *et al.*, 1996). As seen in the benthic fauna, there may be shifts in hyporheic macroinvertebrate community structure. Boulton and Stanley (1995), for example, found that the invertebrate assemblage of an intermittent desert stream changed from being Copepoda and Chironomidae (Diptera) dominated to having a predominance of smaller meiofauna (including micro-Turbellaria, Ostracoda and Nematoda).

2.5 Invertebrate persistence during disturbance events

Invertebrates play a central role in the functioning of lotic freshwaters, supporting food webs (and in particular fish production), processing organic matter, transporting energy and altering the sediment structure through their activity (Covich *et al.*, 1999; Moore, 2006). Benthic macroinvertebrates are also routinely exploited by regulatory bodies as biomonitors to assess the 'health' of running waters (Berkman *et al*. 1986; Wright *et al.*, 2000; Statzner *et al.*, 2001). Their persistence during adverse instream conditions is therefore essential from both ecological and anthropogenic perspectives, and is either achieved through resistance (the ability to survive a disturbance) and/or resilience (the ability to recover after a disturbance; Lake and Barmuta, 1986; Lake, 2000). In rivers that experience predictable, seasonal spate, low flow or drying disturbances, the resident fauna should have evolved adaptations that confer resistance and/or resilience to the recurring conditions (Lytle and Poff, 2004). In contrast, the unpredictable occurrence of a disturbance event in any system may result in more marked reductions in abundance and taxonomic diversity (Boulton, 2003). Invertebrate resistance and/or resilience can be achieved through various adaptations, which during droughts include physiological, life history and/or behavioural strategies (Humphries and Baldwin, 2003), but during spates are largely restricted to behavioural adaptations.

2.5.1 Physiological adaptations to streambed drying

Physiological adaptations include dehydration tolerance at the egg, larval or adult stage of the lifecycle (Williams, 1996). Brock *et al*. (2003), for example, noted the importance of the dehydration tolerant egg bank for invertebrates resident in temporary wetlands, and a diapausing egg stage has also been reported for species of Simuliidae, Chironomidae, Capniidae (Plecoptera; Gray, 1981), Planariidae (Turbellaria; Kolasa, 1991), Limnephilidae (Trichoptera; Wissinger *et al*., 2003) and Oligochaeta (Kenk, 1949; Williams, 2006) in temporary streams. Dehydration tolerant larvae include those of the mayfly *Siphlonurus typicus*, (Kosnicki, 2005), the horsefly *Tabanus dorsifer* (Gray, 1981), several Chironomidae (Cranston and Nolte, 1996; Chou *et al*., 1999), and early instar limnephilid caddisflies (Wissinger *et al*., 2003). Several Coleoptera and Hemiptera species are able to tolerate dehydration as adults (Williams, 1996), for example *Helophorus brevipalpis* (Coleoptera) may complete its lifecycle entirely within the terrestrial environment (Landin, 1980); whilst such taxa are also common in submerged habitats, they should be considered semi-aquatic.

2.5.2 Life history adaptations to streambed drying

Life-history adaptations are also common strategies that promote survival of temporary stream inhabitants. These adaptations typically involve the synchronisation of life history events with appropriate stages of a stream’s typical hydrological regime (Lytle and Poff, 2004). Desiccation resistant eggs may remain dormant during a dry period, only hatching following the return of surface flow. In addition, fast (days to weeks) development allows lifecycles to be completed prior to recurrent flow cessation in temporary waters, as reported for various chironomids, the genus *Prosimulium* (Simuliidae; Kownacki, 1985) and several mayflies (Gray, 1981). Emergence of adult insects prior to the dry phase also promotes survival in intermittent streams, as reported for several genera of Limnephilidae caddisflies, which shelter in terrestrial habitats including caves before returning to the stream to lay their eggs after the return of surface flow (Williams, 1996; Smith *et al*., 2003). Such adaptations, however, offer little protection against supra-seasonal drought, and may also be ineffective if a regular drying event occurs earlier or later than is typical. The most effective life history adaptations for persistence during unpredictable events are perhaps 'bet-hedging' strategies (Lytle and Poff, 2004), for example *Dinocras* stoneflies produce egg clutches that hatch asynchronously (Frutiger, 1996).

2.5.3 Behavioural adaptations to instream disturbance

Behavioural adaptations that promote survival during hydrological disturbances centre on the use of various physical habitat refugia. According to Lancaster and Belyea (1997, p. 222), refugia are 'places… where the negative effects of disturbance are lower than in the surrounding area', and these refugial places facilitate both resistance and resilience of invertebrate communities (Lake, 2000). The ability of a habitat to function as a refugium depends on the nature of the disturbing forces: during streambed drying, refugia are areas that retain free water or high levels of moisture (Humphries and Baldwin, 2003), whilst during spates, refugia are areas where hydraulic stress does not increase significantly (Lancaster and Hildrew, 1993a).

During spates, slow-flowing refugia include dead zones (where shear stress remains low at high discharge; Lancaster and Hildrew, 1993a, 1994; Lancaster, 1999; Rempel *et al*., 1999), inundated floodplains (Townsend *et al*., 1997; Matthaei and Townsend, 2000), stable substratum particles (Townsend, 1989; Cobb *et al*., 1992; Matthaei *et al*., 2000), microform bed clusters (organised groups of surface stones that are resistant to entrainment; Reid *et al*., 1992; Matthaei and Huber, 2002), woody debris (Palmer *et al*., 1996) and riparian vegetation (Robinson *et al*., 2004). During streambed drying, refugial habitats that retain free water or high levels of moisture include crayfish burrows, woody debris, algal mats, large substratum particles and receding pools (Boulton, 1989; Boulton and Lake, 2008; Stubbington *et al*., 2009b). There is therefore little overlap of suitable refuges during high and low flow disturbances and many proposed refugia are localised in occurrence. An exception to this, however, is the subsurface sediments of the hyporheic zone, which may retain free water during surface drying and which may remain stable and slow-flowing during spates.

2.6 The hyporheic zone habitat

2.6.1 Defining the hyporheic zone

Building on early observations of invertebrate fauna in the subsurface sediments (Karaman, 1935; Chappuis, 1942), Orghidan (1959, 2010) was the first to use the term 'hyporheic' to describe the sediments beneath the streambed. Sixty years later, no single definition of the hyporheic zone has been widely accepted (White, 1993; Boulton *et al*., 1998), and in particular the upper boundary of the zone has not been well characterised (Adkins and Winterbourn, 1999). These difficulties in definition reflect the dynamic nature of the zone (Vervier *et al*., 1992; Fraser and Williams, 1998) and the importance of contributions from both groundwater and surface water in determining its character (Brunke and Gosner, 1997; Boulton *et al.,* 1998). In addition, relevant upper and lower boundaries may differ depending on the perspective (e.g. ecological, biogeochemical or hydrological) and research aims of a particular project. For the purposes of the current study, it is sufficient to use a general definition of the hyporheic zone as a spatially fluctuating ecotone that comprises saturated sediments that exchange water with both the overlying surface water and underlying groundwater (White, 1993; Malard *et al*., 2002: Williams *et al*., 2010; Krause *et al*., in press). An ecological assumption inherent within such a definition is the potential of the hyporheic zone to be used as a habitat by both predominantly benthic invertebrates (occasional hyporheos) as well as permanent hyporheic residents (permanent hyporheos, *sensu* Williams and Hynes, 1974) including groundwater specialists.

2.6.2 The hyporheic zone in a landscape context

Conceptualisation of streams as three-dimensional spatial entities was established by Godbout and Hynes (1982), referring to the longitudinal, lateral and vertical linkages between the surface channel and its surrounding catchment. This was later formalised by Ward (1989), who incorporated time as a fourth dimension, recognising the temporal variability in all connections between ecosystem components. In addition to vertical linkages with the surface channel above and the groundwater below, the 'hyporheic corridor' (*sensu* Stanford and Ward, 1993) also includes areas in lateral connection with the hyporheic zone, such as the parafluvial zone and the alluvial aquifer (Boulton *et al.*, 1998; Figure 2.3); these connections may all be affected by temporal variability in hydrological conditions (Malard *et al.*, 2002).

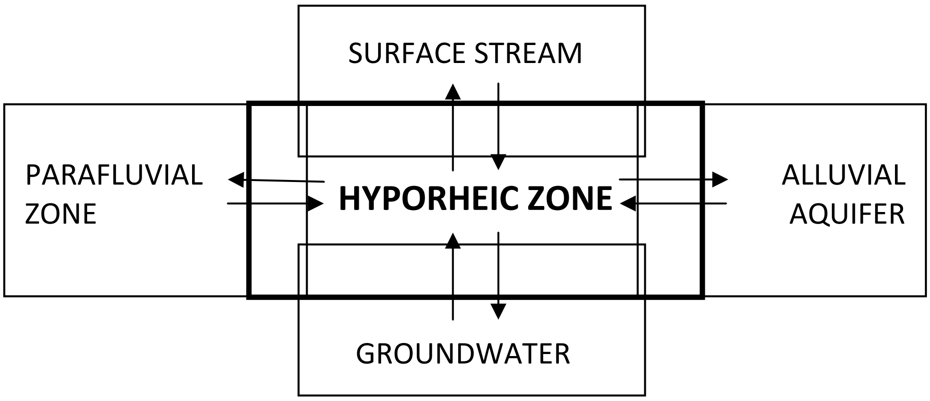


Figure 2.3: Schematic interpretation of the potential hydrological linkages between the hyporheic zone and its surroundings.

The vertical extension of the hyporheic zone is very variable. Some lotic waters lack any significant hyporheic zone, such as in constrained mountainous streams (Stanford and Ward, 1993) and in other reaches underlain by bedrock (Gooseff *et al.*, 2005; Stubbington *et al.*, 2009b). At the other extreme, Stanford and Ward (1988) demonstrated that large alluvial rivers may have deep hyporheic sediments, the average depth of the Flathead River hyporheic zone being 10 m. Intermediate between these values, Palmer *et al.* (1992) recorded a shallow hyporheic zone of ~50 cm beneath a fourth-order stream in northern Virginia, whilst Boulton and Stanley (1995) measured depths of ≥1 m in an intermittent desert stream; many ecological investigations in the hyporheic zone have to date focussed on such shallow sediments (i.e. 10 cm to 1 m). The lateral extent of the hyporheic zone is also temporally variable, and parafluvial zones (which are unsaturated during base flow) can become hyporheic zones as flow increases, whilst typically saturated hyporheic sediments may become parafluvial zones during low flows (Datry and Larned, 2008; Figure 2.2(i-ii)).

2.6.3 The ecological importance of hydrologic exchange

Hydrologic exchange between ecosystem components (e.g. the surface stream and the hyporheic zone) occurs due to the presence of a hydraulic gradient (a difference in water pressure) under conditions of suitable hydraulic conductivity (a measure of resistance to flow caused by porous substrata). Heterogeneity in sediment composition and porosity causes variation in both these hydraulic parameters (Jones and Holmes, 1996), along with local factors such as the depth of the groundwater table (Williams, 1993), and landscape-scale hydrogeological and climatic factors (Hahn, 2006). If the hydraulic gradient or conductivity is insufficient, however, then hydrologic exchange cannot occur and the linkage between the surface stream and the hyporheic zone is lost. Such conditions occur in impervious reaches, reflecting the nature of the underlying substrate (Hill *et al*., 1998; Jones, 2002) or may occur due to the infiltration of fine material into interstices and subsequent colmation (Schälchli, 1992; Brunke, 1999; Pretty *et al*., 2006; Sarriquet *et al*., 2007). In contrast, porous, coarse-grained sediments promote high levels of hydrologic exchange (Munn and Meyer, 1988; Packman and Salehin, 2003; Pretty *et al*., 2006).

Patterns of hydrologic exchange between surface waters and the hyporheic zone have been examined in relation to streambed topography and associated changes in hydraulic pressure. Typically, downwelling water infiltrates the sediments at the head of shallow riffles, where a decrease in depth creates a zone of high pressure which forces water into the sediments (Figure 2.4). Accordingly, upwelling water tends to enter the surface stream downstream of riffles as water depth increases and surface pressure decreases (Harvey and Bencala, 1993; Brunke and Gosner, 1997; Hill *et al*., 1998; Franken *et al*., 2001; Malard *et al*., 2002) (Figure 2.4). These typical patterns are termed bedform-driven hydrologic exchange. However, such exchange may not occur in reaches lacking a definite riffle-pool sequence (Pretty *et al*., 2006), or the reverse pattern may occur, with upwelling water at the riffle head and downwelling water at the tail (e.g. Dole-Olivier and Marmonier, 1992b; Dole-

Olivier *et al.*, 1997), for example due to changes in sediment permeability (Malard *et al.*, 2002). In addition, streambed topography may be altered by instream features such as woody debris dams or macrophyte stands, resulting in localised patches of upwelling or downwelling water (Baxter and Hauer, 2000; White and Hendricks, 2000).

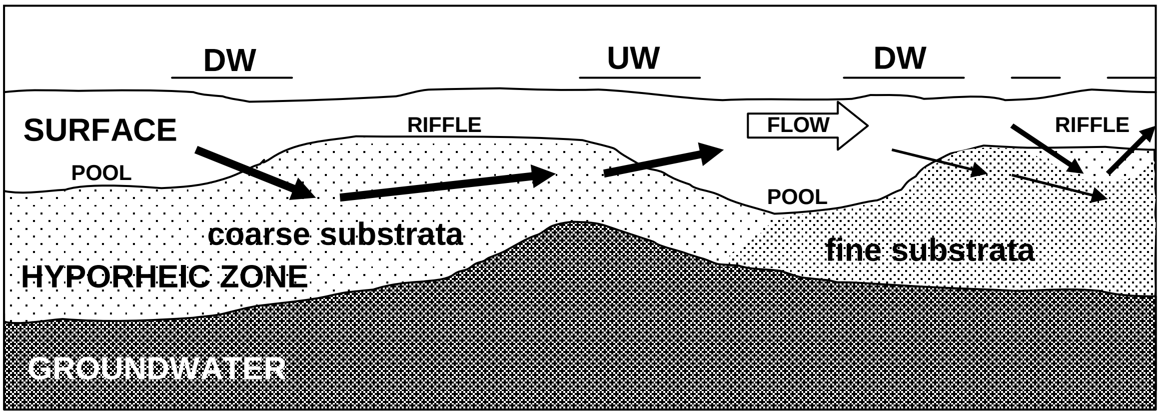


Figure 2.4: A typical longitudinal stream reach, illustrating bedform-driven hydrological exchange between the stream surface and the hyporheic zone. Arrows indicate the strength and direction of hydrologic exchange. DW = downwelling zones, UW = upwelling zones (adapted from Franken *et al.*, 2001; Malard *et al.*, 2002).

The ecological integrity of the hyporheic zone is dependent on unimpeded hydrologic exchange with both the surface stream and the underlying groundwater (Brunke and Gosner, 1997; Hancock, 2002; Figure 2.2). Through this exchange, the hyporheic zone transports water, nutrients, oxygen, organic matter and organisms between ecosystem components. Upwelling groundwater often has high nutrient concentrations (Stanford and Ward, 1988; Wondzell and Swanson, 1996), and in particular, bacteria-driven nitrification in sufficiently well-oxygenated sediments causes upwelling water to be a source of nitrate to the surface stream (Ford and Naiman, 1989: Triska *et al.*, 1993; Holmes *et al.*, 1994; Valett *et al.*, 1994; Jones *et al.*, 1995b). In nutrient limited surface waters, this upwelling of nitrate-rich hyporheic water can fuel productivity (Jones and Holmes, 1996), observed as patches of high algal biomass (Stanford and Ward, 1988; Coleman and Dahm, 1990; Valett *et al.*, 1994). In contrast, where anoxic interstitial waters cause denitrification processes to dominate, the hyporheic zone can also act as a nitrate sink (Pinay *et al.*, 1994; Duff and Triska, 2000). Downwelling surface water typically provides the hyporheic sediments with dissolved oxygen (Grimm and Fisher, 1984; Jones *et al.*, 1995a, b) and inputs of dissolved and particulate organic matter (Rutherford and Hynes, 1987; Ford and Naiman, 1989; Vervier and Naiman, 1992; Findlay *et al*., 1993), which combine to promote hyporheic respiration (Jones *et al*., 1995a). High concentrations of interstitial organic matter stimulate the growth of biofilms, which cover sediment grains and detrital surfaces, and consist largely of bacteria within a matrix of extracellular polymers (Bärlocher and Murdoch, 1989). These biofilms are protein-rich (Leichtfried, 1998) and are therefore an important food source for hyporheic invertebrates (Bärlocher and Murdoch, 1989; Williams, 1993; Brunke and Gosner, 1997).

2.7 The hyporheic zone as an invertebrate refugium

The hyporheic zone's capacity to act as a refugium for predominantly benthic invertebrates was first observed by Orghidan (1959, 2010) during a period of freezing in the surface stream. This observation was later followed by the formal proposition of the zone's refugial role in the Hyporheic Refuge Hypothesis (HRH; Williams and Hynes, 1974), which states that benthic invertebrates will migrate into deeper sediments to escape some adverse condition in the surface stream. These adverse conditions can take a wide variety of forms, and include physical disturbances such as freezing, streambed drying, spates and warm water, as well as biotic factors such as predation, cannibalism and competition. By temporarily migrating into the hyporheic zone, benthic invertebrates may increase their probability of surviving the adverse condition. Following the return of favourable conditions in the surface sediments, both active migrants and those surviving through passive refugium use (i.e. those invertebrates protected due to their passive presence within the hyporheic zone) are a source of benthic zone recolonists; the hyporheic refugium can therefore promote both invertebrate resistance and resilience.

Despite receiving more attention than other potential refugia, particularly with respect to the hydrologic extremes of spates and drying, evidence for the HRH remains equivocal and the factors controlling refugium use have not been adequately characterised. In addition, little research has considered the hyporheic zone as a refugium during low flow conditions, particularly during prolonged flow recessions, despite the potentially significant effects of such conditions on benthic fauna (section 2.4.3). Evidence supporting and contradicting the hyporheic zone during the hydrological conditions of relevance to the current study (spates, low flows and streambed drying) is outlined below.

2.7.1 The hyporheic refugium during spates

It was the observation that invertebrates occurred deeper in the hyporheic sediments during spate flows (thus reducing their risk of displacement) that led to the formulation of the HRH (Williams and Hynes, 1974), and more recently, the more specific Flood Refuge Hypothesis (Boulton *et al*., 2004). Several studies have contributed evidence of active migrations into the hyporheic zone during either natural or experimental high flows (Table 2.4), for taxa including hydrobiid snails, leptophlebiid mayflies, leptocerid caddisflies (Holomuzki and Biggs, 2000), chironomid larvae, elmid beetles (Marchant, 1995) and many other taxa (Dole-Olivier and Marmonier, 1992a; Dole-Olivier *et al*., 1997; Bruno *et al*., 2009). However, active migrations are often restricted to certain benthic species or groups (Marchant, 1995; Lancaster, 2000) or may not be observed at all (Imbert and Perry, 1992; Gayraud *et al*., 2000; Olsen and Townsend, 2005). In many cases, this lack of refugium use can be attributed to inappropriate hyporheic habitat conditions, such as clogging of interstitial spaces by fine sediments (Olsen and Townsend, 2005), or ‘wash-out’ of invertebrates in upwelling zones (Dole-Olivier *et al*., 1997). In other cases disturbance-related parameters appear responsible, in particular the rapid onset of an increase in discharge (Imbert and Perry, 1992; Gayraud *et al*., 2000). Additional evidence suggesting the importance of disturbance characteristics in determining refugium use is provided by studies reporting a reduction in the abundance of permanent hyporheos following a spate (Olsen and Townsend, 2005; Hancock *et al*., 2006); in such cases, disturbance magnitude is sufficient to affect hyporheic as well as benthic sediments and biota.

Table 2.4: Evidence of active, passive and no use of the hyporheic zone as a refugium by benthic invertebrates during spates

SITE DESCRIPTION	TYPE OF REFUGIUM USE – AND EVIDENCE	EXPLANATION	REFERENCE
Intermittent stream, Indiana, USA	**ACTIVE** – benthic taxa present in deeper sediments after spate	-	Clifford, 1966
Speed River, Ontario, Canada	**ACTIVE** – benthic taxa most abundant in deeper sediments after spate	-	Williams and Hynes, 1974
Bypassed section of Rhône River, France	**ACTIVE** – strong relationship between spate magnitude and vertical distribution of benthic fauna	-	Dole-Olivier and Marmonier, 1992a
Acheron River, Australia	**ACTIVE** – Chironomidae, Elmidae, Hydracarina & Copepoda migrated deeper in response to increase in discharge	-	Marchant, 1995
Bypassed section of Rhône River, France	**ACTIVE** – benthic taxa in deeper sediments in downwelling zones after low and medium magnitude floods	-	Dole-Olivier *et al*., 1997
Experimental flow tank	**ACTIVE –** Hydrobiidae, Leptophlebiidae and Leptoceridae migrated to deeper layers during increases in flow	-	Holomuzki and Biggs, 2000
Lake outflow, Ontario, Canada	**PASSIVE** – Hyporheic abundance of Simuliidae unaffected by spate, whilst benthic abundance declined.	?	Giberson and Hall, 1988
Goose Creek, Virginia, USA	**PASSIVE** – little support that meiofauna actively migrate to deeper sediments	Sandy substrate	Palmer *et al*., 1992
Cobble-bed sub-Alpine stream, France	**PASSIVE** – invertebrate densities did not increase in deeper sediments after an experimental increase in discharge	Rapid spate onset	Gayraud *et al*., 2000
Subtropical river, Australia	**PASSIVE** - epigean water mites (Hydracarina) were not more abundant in deeper sediments after experimental spate	Low spate magnitude	Boulton *et al*., 2004
Kye Burn, New Zealand	**PASSIVE** – no evidence of invertebrate migration in response to spates	Fine sediments	Olsen and Townsend, 2005
Alpine stream, Italy	**PASSIVE** – hyporheic invertebrate abundance and diversity reduced at hydropeaking-impacted sites	Disturbance frequency	Bruno *et al*., 2009
Bypassed section of Rhône River, France	**NONE** – Benthic taxa drifted rather than entering the hyporheic zone during high magnitude floods	High spate magnitude, unstable sediments	Dole-Olivier *et al*., 1997
Gravel-bed experimental stream	**NONE –** experiments showed that invertebrates did not migrate in response to abrupt or stepwise flow increases	Rapid spate onset	Imbert and Perry, 1999

Table 2.5: Evidence of active, passive and no use of the hyporheic zone as a refugium by benthic invertebrates during streambed drying

SITE DESCRIPTION	TYPE OF REFUGIUM USE – AND EVIDENCE	EXPLANATION	REFERENCE
Intermittent stream, Indiana, USA	**ACTIVE** – Isopoda, Amphipoda and Coleoptera burrowed into moist interstitial spaces	-	Clifford, 1966
Intermittent streams, Australia	**ACTIVE –** 35 % of benthic taxa sought refuge in deeper sediments		Boulton *et al*., 1992
Arid-zone intermittent stream, Australia	**ACTIVE –** benthic invertebrates moved deeper into hyporheic zone in response to drying	-	Cooling and Boulton, 1993
Intermittent desert stream, Arizona, USA	**ACTIVE** – invertebrate abundance decreased in shallow sediments and increased in deeper sediments as water table declined	-	Clinton *et al*., 1996
Intermittent stream, New York, USA	**ACTIVE** – increase movements of invertebrates into hyporheic zone during drying	-	Delucchi, 1989
Intermittent headwater stream, Ontario, Canada	**PASSIVE** – Hydropsychidae larvae observed in moist interstitial spaces	-	Imhof and Harrison, 1981
Intermittent streams, Arizona, USA	**PASSIVE** – 69 % of benthic taxa present in hyporheic zone during dry phase	-	Boulton *et al*., 1992
Appalachian headwater streams, USA	**PASSIVE** – increased survival of benthic invertebrates in deeper sediments, but no active migrations	-	Griffith and Perry, 1993
Sub-Alpine river, Italy	**PASSIVE –** *Agabus paludosus* present in deep sediments during the dry phase	-	Fenoglio *et al*., 2006
Intermittent streams, Algeria	**PASSIVE** – a few benthic taxa survived the dry phase at low abundance in the hyporheic zone	Compacted fine sediments limited interstitial space	Gagneur & Chaoui-Boudghane, 1991
Intermittent streams, California, USA	**PASSIVE –** abundance of benthic invertebrates did not increase in the hyporheic zone during the dry phase	-	Del Rosario and Resh, 2000
Intermittent streams, Australia	**NONE** – only permanent hyporheos common in hyporheic zone during the dry phase	High streambed temperature	Boulton, 1989
Headwater stream, S Carolina, USA	**NONE -** benthic invertebrates not present	Anoxia, sandy substrate	Smock *et al*., 1994
Intermittent stream, Arizona, USA	**(ALMOST) NONE** – very few benthic taxa survive dry phase in hyporheic zone	Hyporheic zone dried	Boulton and Stanley, 1995
Intermittent wadi, Algeria	**NONE** – benthic invertebrates not present	Baked fine sediment crust resulted in anoxia	Belaidi *et al*., 2004

In almost all field studies that have reported no evidence for use of the hyporheic refugium (i.e. no active vertical migrations) during spates, benthic invertebrates have nonetheless been present in the hyporheic zone after the event, but at low abundance (Giberson and Hall, 1998; Boulton *et al*., 2004; Olsen and Townsend, 2005). Such observations highlight the additional importance of passive use of the hyporheic refugium in promoting invertebrate resistance and resilience during spates; even if only a few invertebrates survive, these individuals are an important source of recolonists of the benthic sediments after the spate has ended. Giberson and Hall (1988), for example, noted that the abundance of Simuliidae larvae was significantly reduced in the surface stream following a spate in a Canadian lake outflow stream whilst hyporheic abundance remain unchanged (Table 2.4); this equated to an increase in the hyporheic proportion of the population, which could potentially migrate to the surface stream following the return of favourable flow conditions.

2.7.2 The hyporheic refugium during low flows

Little research has examined the use of the hyporheic zone refugium by benthic invertebrates during low flows (James *et al*., 2008; James and Suren, 2009; Stubbington *et al*., 2009a; Wood *et al*., 2010), with the longest uninterrupted period of flow recession studied continuing for two months (Stubbington *et al*., 2009a; Wood *et al*., 2010); this highlights the difficulties in examining temporally unpredictable hydrological conditions (Boulton and Lake, 2008). None of these few studies considering use of the hyporheic zone during low flows has directly linked a decline in discharge to an increase in the hyporheic abundance of benthic taxa. One study (Stubbington *et al*., 2009a; Wood *et al*., 2010) did note a significant increase in the hyporheic abundance of the dominant benthic amphipod, *Gammarus pulex*, however this coincided which particularly high air and water temperatures and not the lowest discharges; this highlights the additional role of the hyporheic zone as a thermal refugium (Evans and Petts, 1997; Dewson *et al*., 2007a).

James *et al*. (2008) hypothesized that benthic invertebrates would migrate into the hyporheic zone during low flows if habitat contraction concentrated invertebrates into a smaller submerged area, thus increasing biotic interactions (e.g. predation and competition) in the surface sediments. Such predictions are valid, given the general observation that prey taxa move into lower-risk habitats in response to an increase in predation pressure (Sih, 1987), and specific experimental evidence demonstrating that mobile benthic taxa such as *Gammarus pulex* (Amphipoda: Crustacea) migrate into habitat with smaller interstitial spaces in response to an increased risk of cannibalism (McGrath *et al*., 2007). Additional support for this hypothesised role of the hyporheic zone as a low flow refugium comes from the zone's established function as a nursery for vulnerable early instars of various Insecta (Giberson and Hall, 1988; Puig *et al*., 1990; Jacobi and Cary, 1996) and its proposed role as a refugium from high-risk biotic conditions in the surface sediments, regardless of flow conditions (Marmonier and Creuzé des Châtelliers, 1991; Dole-Olivier et al., 1997). However, neither James *et al*. (2008) nor other studies have reported any evidence supporting this hypothesis. The hyporheic zone potentially has disadvantages for benthic invertebrates, including low light levels, impeded mobility through interstitial spaces and low abundance of suitable food resources; in addition, during low flows, integrity of the hyporheic habitat may be compromised by clogging of interstices with deposited fine sediments; these drawbacks appear to outweigh the refugial benefits offered by the hyporheic zone during low flows (James *et al*., 2008).

2.7.3 The hyporheic refugium during streambed drying

The hyporheic zone's ability to act as a refugium following the loss of surface water relies on the availability of free water, or at least high humidity, in interstitial spaces. Where this criterion has been met, several studies have used increases in hyporheic abundance to infer vertical migrations of benthic invertebrates into deeper sediments during the dry phase (Delucchi, 1989; Cooling and Boulton, 1993; Clinton *et al*., 1996; Table 2.5), indicating active shelter-seeking behaviour (Wood *et al*., 2010). In addition, several other investigations, whilst not inferring active migrations, have nonetheless found passive inhabitation of the hyporheic sediments to promote survival in a range of invertebrates including *Lirceus fontinalis* (Isopoda: Crustacea), *Crangonyx forbesi* (Amphipoda: Crustacea; Clifford, 1966), *Hydropsyche* spp. (Hydropsychidae: Trichoptera; Imhof and Harrison, 1981), *Agabus paludosus* (Dytiscidae: Coleoptera; Fenoglio *et al*., 2006) and many other taxa (Boulton *et al*., 1992; Griffith and Perry, 1993; Del Rosario and Resh, 2000). In contrast, a number of similar studies have noted no refugium use, either active or passive, which has generally been attributed to an inadequacy in the habitat provided by the hyporheic zone, for example interstitial anoxia (Smock *et al*., 1994; Belaidi *et al*., 2004), or the loss of interstitial free water (Boulton and Stanley, 1995; Table 2.5). Most research considering the hyporheic refugium during streambed drying has been conducted in arid and Mediterranean climates and comparative information from temperate zones remains scarce (Table 2.5).

2.7.4 Combined factors influencing use of the hyporheic refugium

The equivocal evidence for use of the hyporheic refugium during spates and streambed drying and the absence of expected refugium use during low flows have been attributed in part to inadequacies in the hyporheic zone environment, for example anoxia following drying, and unstable sediments and upwelling water during spates. However, given the notorious heterogeneity of instream habitats, it is unlikely that environmental factors alone dictate hyporheic refugium use in all instream areas (Lancaster and Belyea, 1997; Lancaster, 2008), and several studies have stressed the additional importance of disturbance-related parameters such as disturbance magnitude and rate of onset. Despite the potential importance of a combination of environmental and disturbance-related factors in controlling refugium use in a particular situation, little research has considered benthic invertebrate use of the hyporheic sediments during a prolonged period comprising multiple hydrological or hydrologically-mediated adverse conditions.

2.8 Summary

Recognition of the four-dimensional, spatiotemporally dynamic nature of lotic ecosystems has provided the impetus for research exploring the longitudinal, lateral and vertical linkages of surface streams. However, research into the vertical dimension has lagged behind that considering other linkages and the ecology of the hyporheic zone remains a young and evolving sub-discipline. The literature review presented here has highlighted the ecological importance of the hyporheic zone as a refugial habitat for benthic invertebrates during adverse conditions in the surface stream. However, the equivocal evidence for refugium use during spates and streambed drying and the lack of evidence during low flow conditions highlights the significant gaps that remain in our understanding of the zone's refugial role. In particular, a need for medium- to long-term studies of refugium use during sequential, contrasting hydrological conditions has been identified. These research gaps can be mapped onto the aims and objectives of the current research project (section 1.2). In the following chapters, sites are selected (chapter 3) and methodological approaches developed (chapter 4) in an attempt to address these gaps. Following description and discussion of the results obtained (chapters 5 and 6), key findings are integrated with the existing literature (chapter 7) and practical application of the knowledge gained is considered in the wider context (chapter 8).

3. Site selection

3.1 Introduction

This chapter examines the process of site selection, from the rationale governing the choice of river type to the positioning of sampling points at each selected site. The selected study rivers are described in terms of their geology, hydrology and instream habitats, and natural and anthropogenic influences on streamflow are discussed. The results of baseline surveys conducted in each river are presented and used to inform the process of site selection.

3.2 Selection of river systems

3.2.1 General requirements

River systems were required in which to study the response of benthic and hyporheic invertebrate communities to flow variability, including low flows and streambed drying. Groundwater dominated rivers were selected as they are characterised by relatively predictable flow regimes (Sear *et al.*, 1999) and are susceptible to reduced flows as a result of anthropogenic pressures such as groundwater abstractions for public water supply (Petts *et al.*, 1999). Due to the influence of the underlying geological strata on the discharge regime (Frissell *et al.*, 1986; Cannan & Armitage, 1999), a single dominant lithology was required. Karst limestone was selected, as karst rivers are relatively responsive to precipitation (Burt, 1996) and small-scale changes in the underlying lithology can result in alternating reaches of intermittent and perennial flow (Maddock, 1994; Meyer & Meyer, 2000). A minimum of two study rivers was required, to prevent questionable generalisations being made based on site-specific conditions. Financial and time constraints dictated that no more than two rivers could be adequately characterised, particularly due to the highly variable nature of the hyporheic habitat and hyporheic communities over small areas of the river bed (Dole-Olivier *et al.*, 1997; Soulsby *et al.*, 2009).

3.2.2 Characteristics of karst rivers

The term karst is used to describe a particular type of terrain that develops on and within rocks with high solubility and well-developed secondary porosity, and is most common in carbonate rocks such as limestone (Leibundgut *et al*., 1998; Ford & Williams, 2007). Typical features of a karst landscape include sinking streams, caves, sinkholes and emergent springs, and an extensive system of fissures, cavities and conduits beneath the land surface (Bonacci *et al*., 2009).

Karst terrains are characterised by a low drainage density (Jalali *et al*., 2009), and the few surface streams that are present have a distinctive hydrology. Karst streams are often groundwater dominated due to spring inputs from the limestone aquifer, resulting in a stable flow regime in gaining reaches (Sear *et al*., 1999). Equally, fissures and conduits in the karst bedrock can increase transmission losses in losing reaches (Baffault & Benson, 2009), as can mid-channel sinkholes (Hindley, 1965). Alternating gaining and losing reaches can cause spatially variable patterns of flow permanence over small areas (i.e. <1 km; Maddock, 1994; Meyer & Meyer, 2000).

The flow variability observed in karst streams can be altered by human activity. Low flows, which form a natural part of the hydrological regime, can be further reduced by anthropogenic pressures, principally the abstraction of surface water and groundwater; this is a particular problem where the karst aquifer makes a significant contribution to public water supplies (Doerfliger *et al.*, 1999; Johnson *et al*., 1999). Other human activities can have localised effects, for example the mining of mineral deposits within karst rocks can necessitate construction of drainage adits to dewater the mineral field (Younger *et al*., 2002). These ‘soughs’ can continue to divert water from overlying surface streams and the surrounding catchment long after mining activity has ceased (Gunn, 1998). Any activity which lowers the water table can cause an increase in the spatial and temporal extent of streambed drying in intermittent river reaches (Gunn, 1998; Stubbington *et al*., 2009b).

3.2.3 Selection of two karst rivers

In England, the most extensive and best-developed karst landscapes are seen in the Dinantian age Carboniferous limestones of the North, with notable outcrops including the White Peak area of the Peak District in Derbyshire (Farrant & Cooper, 2008). Karst features are also present in the Jurassic limestones in central, southern and eastern England, most notably in the Lincolnshire Limestone area south of Grantham (Hindley, 1965). The surface streams in these regions are therefore suitable systems in which to examine the effects of spatial and temporal flow variability on instream communities.

With guidance from local representatives of Natural England and the Environment Agency regarding flow regime characteristics, two karst streams were selected for detailed investigation of benthic invertebrate community response to flow variability: the River Lathkill in Derbyshire and the River Glen in Lincolnshire (Table 3.1). Both rivers have been the subject of previous hydrological, geomorphological and ecological research, and as a result extensive literature is available to inform continued investigation (e.g. Gunn, 1998; Wood *et al*., 2005 on the River Lathkill; Bickerton, 1995; Maddock *et al*., 1995; Bradbury & Rushton, 1998 on the River Glen).

3.3 The River Lathkill

3.3.1 Introduction

The River Lathkill (53°11.2’N, 1°44.4’W) is the central feature of the Lathkill Dale Site of Special Scientific Interest (SSSI) and forms part of both the Derbyshire Dales National Nature Reserve and the Peak District Dales Special Area of Conservation (JNCC, 2007), which in turn lie within the Peak District National Park. The Lathkill drains a structural basin centred on Monyash, and is the only major river to originate from springs within the limestone outcrop and to discharge only autogenic water (i.e. water which has only been in contact with carbonate rocks; Figure 3.1). The river flows for 8.5 km and has a catchment area estimated as ≤52 km^2 (Bamber, 1951;

Gunn, 1998). During high flows, the Lathkill rises from the Head Cave at an elevation of ~200 m a.s.l., and is fed in its upper reaches by a series of intermittent springs

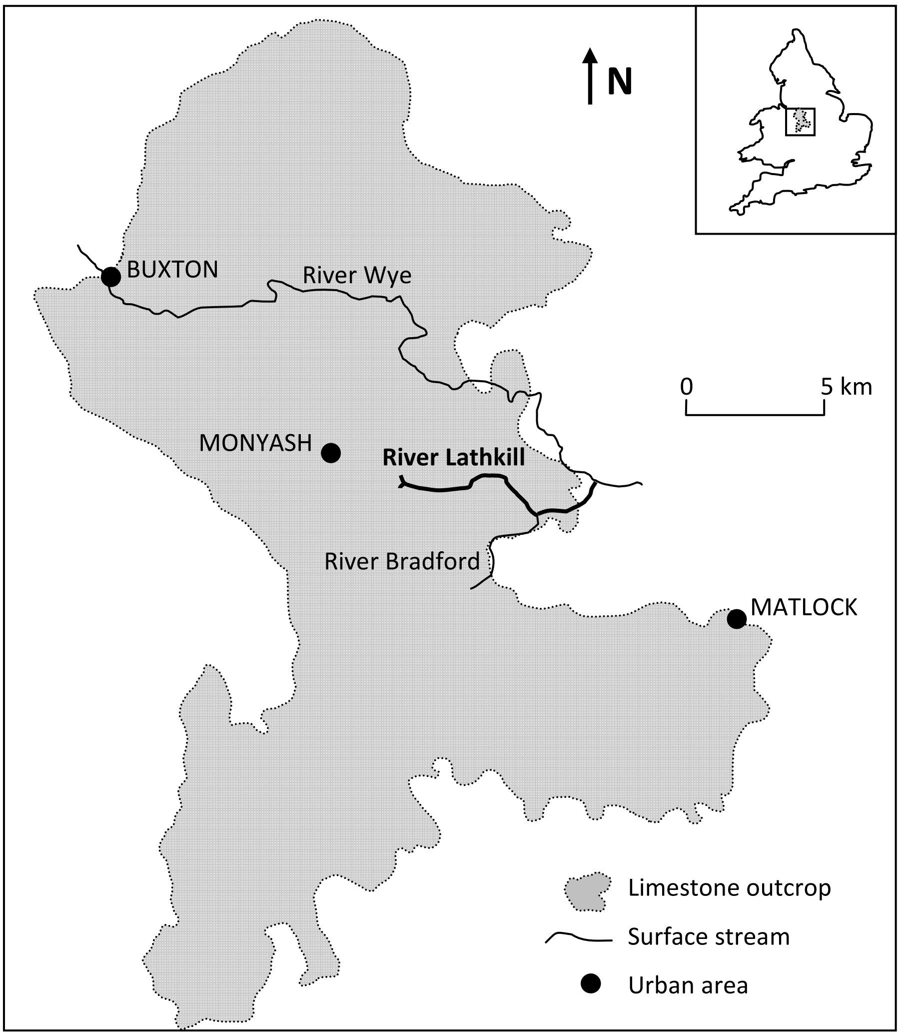


Figure 3.1: Location of the River Lathkill (53°11.2’N, 1°44.4’W) in the limestone outcrop (adapted from Wood *et al.*, 2005).

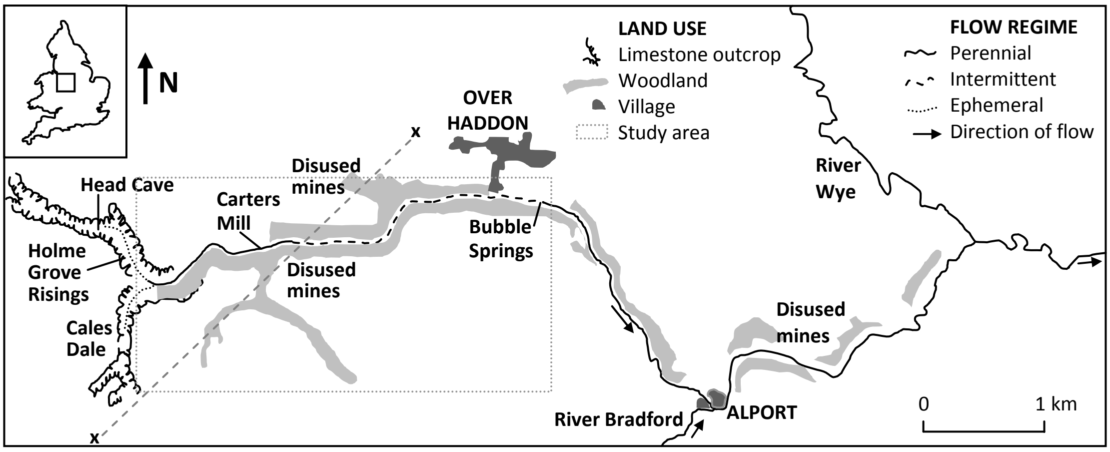


Figure 3.2: Location map of the study area on the River Lathkill, indicating flow permanence regimes and surrounding land use. Line x-x indicates cross section presented in Figure 3.3.

including the Holme Grove Risings (Figure 3.2). The river is joined by an ephemeral tributary at Cales Dale, then descends gradually and merges with its main tributary, the River Bradford, after ~5 km; a further 3 km downstream, the Lathkill reaches an elevation of <110 m a.s.l. at its confluence with the River Wye (Figure 3.2).

3.3.2 Catchment characteristics

The geology of the Lathkill catchment and the surrounding White Peak region is dominated by Carboniferous limestones, which have a highly varied lithology including rapid vertical and lateral facies changes (Gunn, 1998; Figure 3.3). The river valley is incised into Monsal Dale Limestones, which are overlain in parts of the surrounding catchment by Eyam Dale Limestones (Gutteridge, 1991); both formations are of late Dinantian age (British Geological Survey, 2009). Such limestones are compact and well jointed, and as such have low primary porosity (mean effective porosity is 2.9-3.4%; Bell, 1981) and hydraulic conductivity (Gunn, 1998). Groundwater flow is therefore largely restricted to features associated with the secondary porosity, such as joints, fractures and bedding planes, which form complex conduit systems (Gunn, 1998). The secondary porosity may also host major groundwater stores (Atkinson & Smart, 1981), although it has been argued that storage in the overlying unsaturated zone is of greater significance (Gunn, 1986).

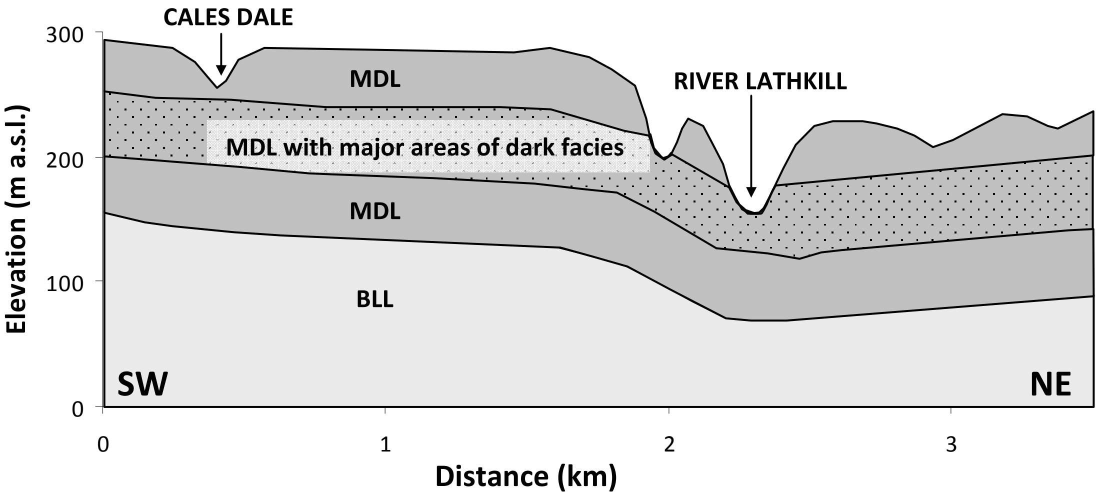


Figure 3.3: Southwest-northeast cross-section (x-x on Figure 3.2) through the River Lathkill catchment. MDL = Monsal Dale Limestone; BLL = Bee Low Limestones (reproduced from Ordnance Survey geological map)

The land surrounding the River Lathkill is a protected nature reserve, and as such is sensitively managed by Natural England and exposed to few current anthropogenic pressures. In the river's upper reaches, the valley sides of the Lathkill Dale are grassland and are grazed by sheep to keep protected habitats in a favourable condition, whilst further downstream the valley sides are densely wooded (Figure 3.2). Water in the Lathkill has long been "famed for its clarity and purity" (Natural England, 1987) and pollution problems remain rare in the catchment (Gunn, 1998).

3.3.3 Local climate

The climate of the Peak District is temperate, with a mean annual air temperature of 8°C, ranging from 1.7°C in January to 14.5°C in July (Wood *et al*., 2005). Mean annual rainfall for the period 1991-2000 (excluding missing data in 1992 and 1994) was 1060 ± 74.8 mm at Monyash (5 km west of the Lathkill at an elevation of 270 m; 53°19.5'N, 1°77.4'W) (BADC, 2009). Precipitation is highest in winter with drier conditions typical during the summer months; for the period 1991-2000, monthly amounts at Monyash peaked in December at 136.0 ± 19.6 mm and were lowest in August at 57.8 ± 10.1 mm (BADC, 2009). Periods of prolonged rainfall tend to occur in winter and early spring, whilst unpredictable high intensity rainfall events are most common in July and August (Met Office, 2009a).

3.3.4 Hydrology

Recharge of the karst aquifer occurs mainly through diffuse autogenic recharge (i.e. direct percolation of rainwater through the overlying soil and superficial deposits; Banks *et al*., 2009), and groundwater inputs from the recharged aquifer dominate surface flow. The response of the surface stream to low and moderate intensity rainfall events is therefore buffered against sharp increases. In contrast, dramatic rises in streamflow may occur if heavy rainfall persists following saturation of the soil and groundwater stores (Stubbington *et al*., 2009b). However, the dominant control governing subsurface flow directions and surface water levels in the Lathkill is not the karst geology, but the legacy of historic mining activity within the catchment (Gunn, 1998), and despite its protected status, the river is affected by the loss of surface flow to extensive underlying networks of soughs. These soughs were constructed in the 18th and 19th centuries to drain mines in the Dale which aimed to exploit the lead-zinc mineralisation within the limestone. Sough construction causes a lowering of the water table and so limits groundwater storage, with consequent declines in spring discharge and therefore streamflow; in addition, baseflow can be diverted underground into soughs.

Today, the headwaters of the Lathkill continue to lose a substantial proportion of spring discharge to Magpie Sough (Bamber, 1951; Gunn, 1998). As a result, the Lathkill is ephemeral downstream of its source (the Head Cave, Figure 3.2) and surface flow is only spatially continuous during the winter months and in response to unpredictable, sustained precipitation inputs (Stubbington *et al*., 2009b). The tributary in Cales Dale shares a common catchment with the Lathkill headwaters and as such has a similar ephemeral flow regime (Gunn, 1998). The duration for which surface water is present in the headwaters gradually increases with progression downstream until spring and tributary inputs result in perennial flow after a distance of ~500 m (Figure 3.2). Further downstream, the Lathkill Dale and Mandale Soughs capture surface flow between Carters Mill and Bubble Springs, resulting in a second intermittent reach that typically loses surface flow between July and September (Gunn, 1998). The river unit of the Lathkill Dale SSSI is currently considered to be in an ‘unfavourable’ condition by Natural England (2009) due to this loss of surface water. Further downstream, groundwater is forced upwards by a basalt barrier, resulting in a continuous supply of water to the surface stream; this area, Bubble Springs, is the perennial head of the River Lathkill.

3.3.5 Instream habitats

Despite its local renown as a pristine stream, the River Lathkill has undergone extensive modifications in both past centuries and recent times. In the mid-1800s the river was channelized in an attempt to maintain surface flow, and the channel remains confined between stone walls along much of its length (Gunn & Dykes, 2000). Despite this, some areas of the river retain a natural pool-riffle sequence, providing a diversity of hydrological habitats for instream communities. In other areas the pool-riffle sequence is interrupted by weirs, also built during the mining era to mitigate against the loss of surface flow (Gunn & Dykes, 2000), and these result in upstream areas of lentic water. Sluice gates have a similar effect on upstream habitat, and remain in operation in one location to maintain habitat for fish during the summer months. In the Lathkill headwaters and the Cales Dale tributary, the substrate comprises exposed karstic bedrock interspersed with areas of fine-grained, organic-rich sediments, and overlain in places by boulder- to granule-sized gravels (*sensu* Wentworth, 1922). Downstream of the Cales Dale tributary confluence (Figure 3.2), the karst geology is overlain by mixed alluvial deposits which increase in depth with progression downstream.

3.4 The River Glen

3.4.1 Introduction

The River Glen (52°42.4’N, 0°22.7’W) rises from the Lincolnshire Limestone ridge to the east of Grantham in south-west Lincolnshire. In its upper reaches, the river comprises two main tributaries, the West Glen and the East Glen, which flow for 39 km and 37 km, respectively, in subparallel north-south aligned valleys before merging to form the River Glen (Figure 3.4); it is the area upstream of this confluence that is the focus of the current research. The East and West Glen together drain an area of 342 km^2 (Maddock, 1994) in a lowland (<80 m a.s.l.), largely agricultural landscape between Grantham in the north and Stamford in the south (Figure 3.4).

3.4.2 Catchment characteristics

The catchment of the River Glen is dominated by Lincolnshire Limestone (Middle Jurassic age), which rests on impermeable Lias clay, and is partly covered by various overlying formations (Figures 3.4 & 3.5). The limestone outcrops throughout the middle reaches of the West Glen, but because the underlying strata dip to the east, the East Glen flows mainly on the overlying formations, and only sporadically on the limestone outcrop. The overlying formations are a complex mix of minor aquifers and aquitards of various lithologies, notably Great Oolite Limestone (limestone with thin marl and clay beds), Upper Estuarine Series (sand, clay, shale and limestone) and glacial drift deposits (diamict, sand and gravel) (Bradbury & Rushton, 1998; Rushton & Tomlinson, 1999; Figure 3.5).

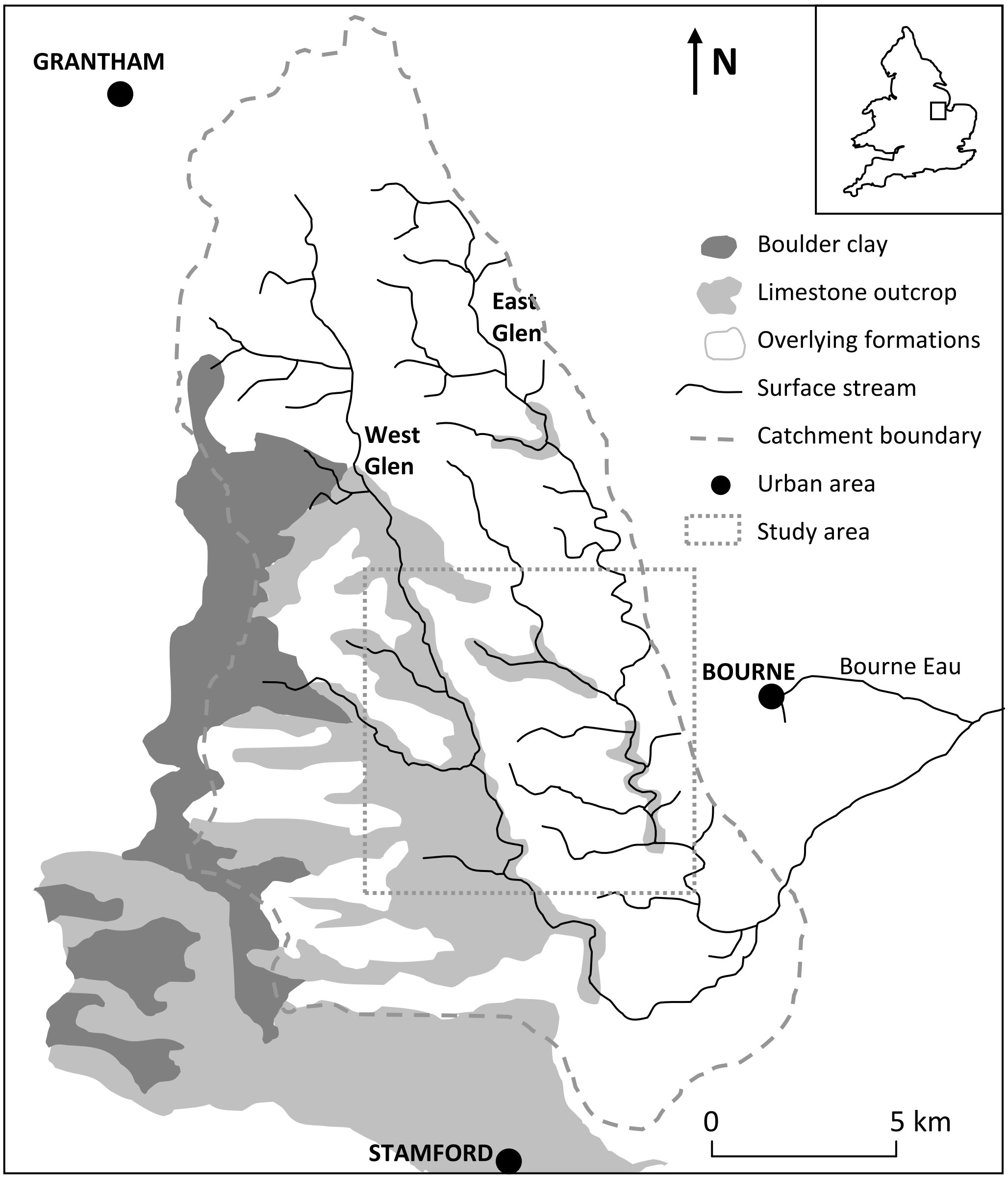


Figure 3.4: Location of the River West Glen and River East Glen catchment (52°42.4'N, 0°22.7'W) in relation to the underlying geology.

The River Glen flows through a rural area, and land use is predominantly agricultural, with managed grassland, urban developments, and woodland occupying only a small proportion of the catchment area (Mattikalli & Richards, 1996; Griffiths *et al.*, 2006). Runoff from agricultural land can have significant impacts on the quality of both surface water and groundwater (Novotny, 2003), with dissolved organic carbon, nutrients (nitrogen and phosphorus) and pesticides amongst the most significant contaminants (Kay *et al.*, 2009). Particular threats affecting the Lincolnshire Limestone aquifer include nitrate pollution in both groundwater (Hiscock *et al.*, 2007; Rivett *et al.*, 2007) and surface water, with concentrations being most elevated where streamflow is provided by surface runoff (Mattikalli, 1996).

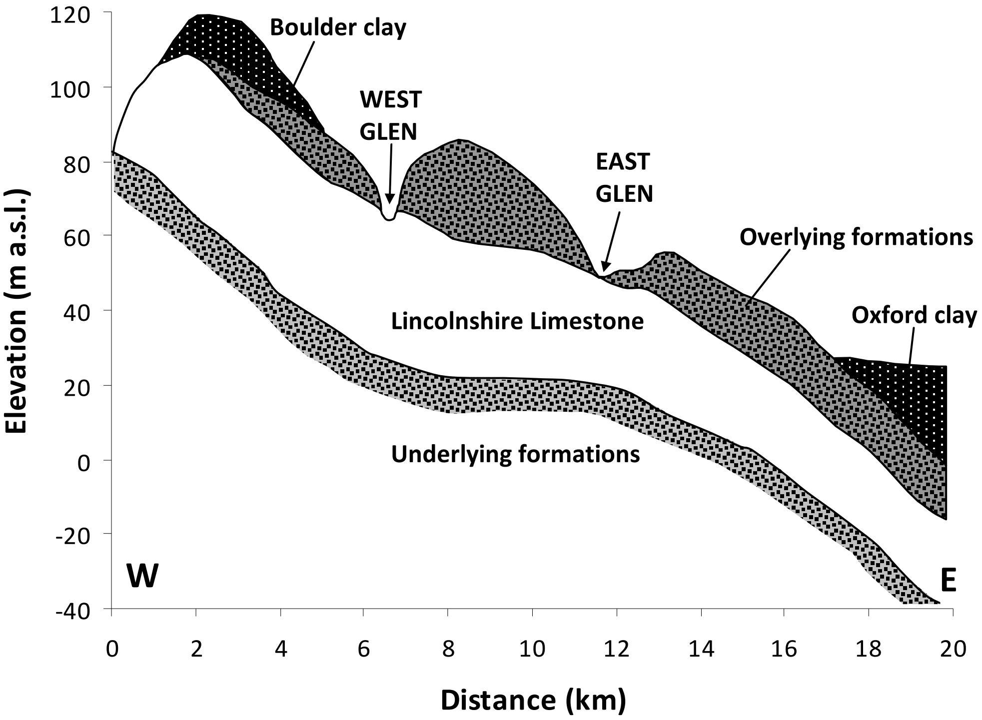


Figure 3.5: Typical west-east cross-section through the West and East Glen catchment (after Bradbury & Rushton, 1998).

3.4.3 Local climate

Mean annual rainfall for the period 1980-2008 (excluding questionable data in 2005 and 2007) was 615 ± 18.1 mm at Carlby, located between the West and East Glen at an elevation of 31 m (52°71.5’N, 0°44.5’W; Figure 3.6) (BADC, 2009). Distribution of rainfall is relatively uniform throughout the year, with mean values varying between 36.4 ± 3.3 mm in February to 61.1 ± 5.6 mm in October (BADC, 2009). The mean annual air temperature in the region is ~10.5 °C, with the coldest conditions experienced in January and February, and the warmest in July and August (Met Office, 2009b); warmer temperatures result in increased evapotranspiration and reduced streamflow during the summer months (Stahl & Hisdal, 2004). Although the region is relatively dry, severe rainfall events can occur at any time of year, and are most common in summer (Met Office 2009b).

3.4.4 Hydrology

Precipitation inputs lead to recharge of the Lincolnshire Limestone aquifer through a complex array of mechanisms, including diffuse autogenic recharge and allogenic recharge *via* runoff in areas where the aquifer is overlain by strata of low permeability (Bradbury & Rushton, 1998). In addition, sinkholes in the Great Oolite Limestone allow direct, rapid autogenic recharge of fissures and other features associated with secondary porosity (Downing & Williams, 1969; Fox & Rushton, 1976). The Jurassic limestones have a high primary porosity (mean effective porosity 14.1-14.4%; Bell, 1981) and a secondary porosity dominated by solutionally-enlarged fissures, resulting in high transmissivity but low storativity (Bottrell *et al.*, 2000; Moncaster *et al.*, 2000).

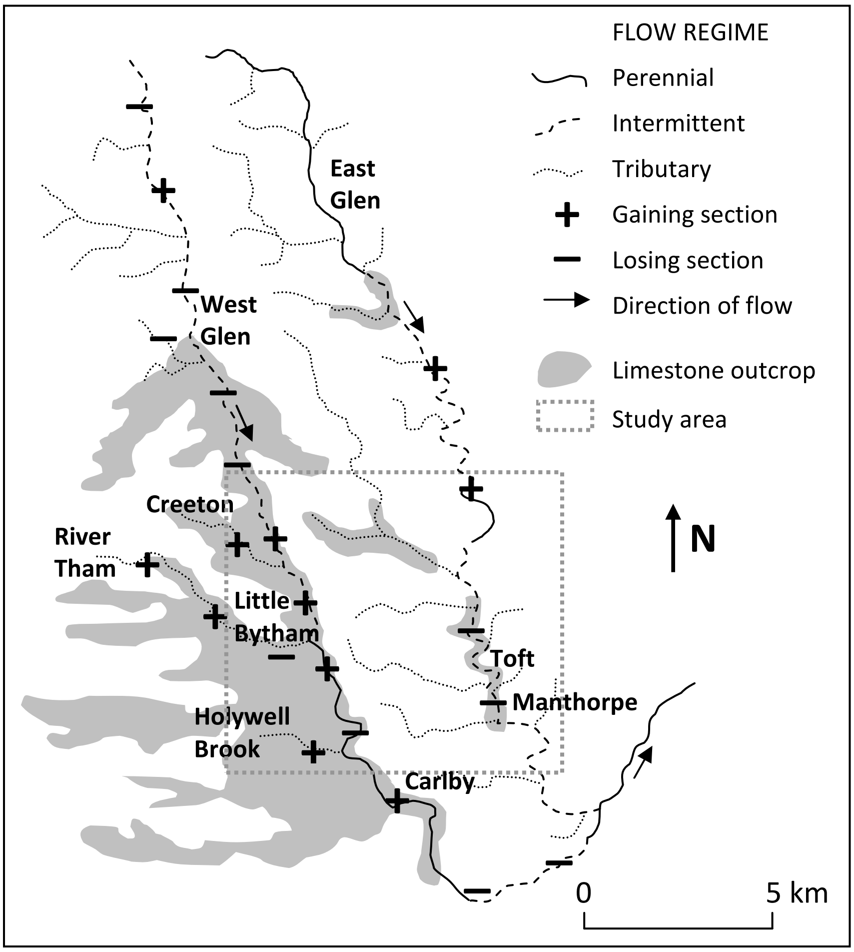


Figure 3.6: The flow regimes of the West Glen and East Glen Rivers; information regarding hydrologic exchange between the surface stream and the underlying aquifer is incomplete for the East Glen.

The topography of the catchment is the primary influence on surface and subsurface flow (Bradbury & Ruston, 1998), with complex surface hydrology resulting from the incision of the river valleys into an alternating sequence of aquifers and low-permeability strata (Rushton & Tomlinson, 1999; Figure 3.6). The West Glen crosses various overlying formations in its upper reaches, and the contribution of runoff to surface flow responds to changes in precipitation falling on impermeable strata whilst minor aquifers provide stable inputs of groundwater. As the river flows onto the limestone outcrop, losing reaches provide recharge to the underlying aquifer, with a corresponding reduction in surface flow (Figure 3.6). Further downstream, groundwater inputs contribute to streamflow directly through upwelling springs in Creeton, in gaining reaches in the Little Bytham area and indirectly *via* the River Tham and Holywell Brook tributaries. These inputs sustain flow in a losing reach upstream of Holywell Brook, although surface flow has disappeared from this reach in recent years due to the appearance of sinkholes (C. Extence, pers. comm.). In its lower reaches, the West Glen flows over the Rutland Formation (sand, clay, shale and thin limestone; Rushton & Tomlinson, 1999) and a proportion of streamflow is lost to underlying minor aquifers. In the upper and middle reaches of the East Glen, streamflow is provided both by surface runoff from impermeable strata and by groundwater spring inputs from minor aquifers. Further downstream, between Toft and Manthorpe, localised outcropping results in the loss of a significant proportion of streamflow to the limestone aquifer, resulting in intermittent flow in these reaches (Figure 3.6).

The Lincolnshire Limestone is a major aquifer in eastern England, and has been extensively developed for public water supply (Barton & Perkins, 1994). As a result, groundwater levels in the Glen catchment have been declining since c. 1940 (Petts, 1990), and the river is considered to be over-abstracted on both the East and West Glen tributaries and downstream of their confluence (Environment Agency, 2007). Consequently, both spring recharge and surface streamflow have declined, and both the West Glen, and in particular the East Glen, now experience streambed drying during the summer months in most years (Maddock, 1994; Bickerton, 1995).

3.4.5 Instream habitats

The River Glen has been considerably altered from its original state in recent centuries, with reports of channelisation at Edenham on the East Glen dating back to 1756 (Maddock, 1994). Maddock (1994) identified a further 24 locations in which the natural, meandering channel had been straightened. In addition, regular dredging and embanking of the channel continues to be carried out to improve land drainage. Such activities have caused degradation of the instream conditions and reduced hydraulic habitat diversity in many reaches (Petts *et al.*, 1992).

Table 3.1: Summary of the River Lathkill and River Glen catchment characteristics

	River Lathkill	***River Glen***
Location	Peak District, Derbyshire, UK	South Lincolnshire, UK
Latitude and longitude	53°11.2'N, 1°44.4'W	52°42.4'N, 0°22.7'W
Length (km)	8.5	West Glen: 39, East Glen: 37
Catchment area (km^2)	51.8	342
Elevation (m a.s.l.)	100-200	< 80
Slope		West Glen: 0.0016 East Glen: 0.0010
Stream order (*sensu* Strahler, 1964)	1st to 2nd	West Glen: 4th, East Glen: 3rd
Mean annual rainfall (mm yr^{-1})	1200	600-630
Mean annual temperature (°C)	8	10.5
Geology	Carboniferous limestones	Jurassic Lincolnshire Limestone
Catchment land use	Grazed grassland, woodland	Arable
Streamflow source	Groundwater only	Groundwater dominated
Anthropogenic influences on streamflow	Water loss to disused mining drainage levels	Abstraction

3.5 Selection of study sites

A critical requirement of any field-based sampling programme is the selection of representative sites (Frissell *et al.*, 1986). To inform the process of site selection for subsequent field sampling, baseline surveys of the benthic invertebrate communities were conducted on both rivers. Sufficient sites (a site being a hydrologically homogeneous area measuring <50 m in length) were selected on each river to encompass the full spatial range of hydrological conditions experienced. The aim of these surveys was to identify suitable sites for the investigation of: i) benthic and hyporheic invertebrate community responses to flow variability; and ii) invertebrate survival following streambed drying.

3.5.1 Selection of baseline survey sites

Nine sites were selected on the River Lathkill in consultation with Philip Bowler of Natural England, who oversees the management of the Lathkill Dale site, and Professor John Gunn of the Limestone Research Group at the University of Birmingham, who has studied the hydrogeology of the Lathkill for several years. These sites comprised: one in the headwaters (1, Figure 3.7); three in the perennial upper reaches (2- 4), which experience exposure of instream topographic high points during dry periods but always retain some surface water; four in intermittent downstream reaches (5-8, the length of the summer dry phase increasing with distance downstream); and, at the downstream extent of the study area, the perennial head of the river (9, Figure 3.7). Further downstream, the river is deep, straight and slow-flowing, and natural flow is regularly interrupted by weirs; the perennial head of the river therefore marks the downstream extent of the area considered by this study.

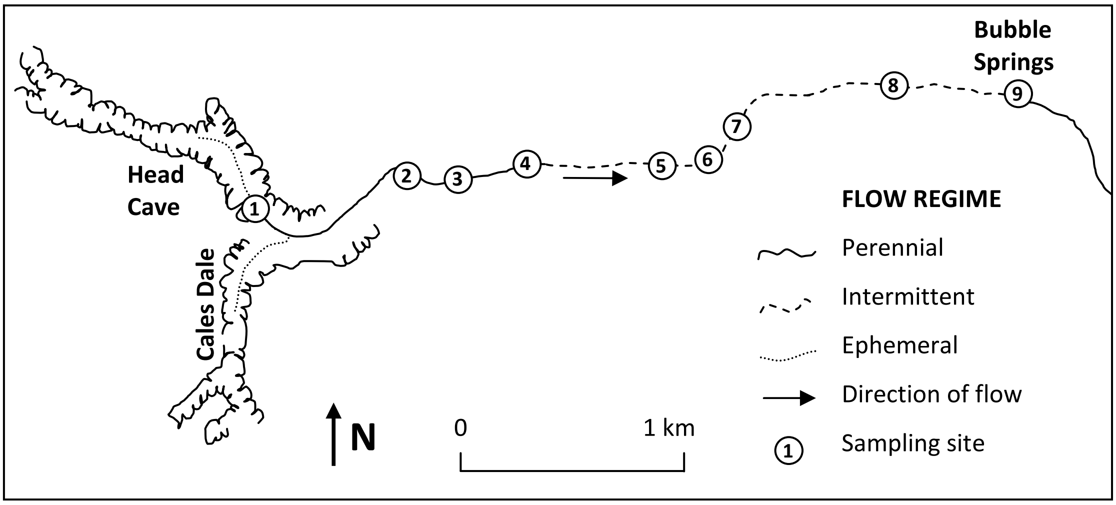


Figure 3.7: Baseline survey sampling points on the River Lathkill.

On the River Glen, sites were selected in consultation with two senior ecologists from the Environment Agency (EA) Anglian region, Dr. Chris Extence and Richard Chadd, who are responsible for regional biomonitoring of river quality. Ten EA routine macroinvertebrate sampling sites were selected; using established EA sampling points was desirable because, firstly, most had several years of ecological data available for later comparison, and secondly, the river was relatively easy to access in these areas. Two sites were subsequently discarded, one due to accessibility issues and the second due to water quality concerns related to sewage effluent inputs. Of the remaining eight sites, five are situated on the West Glen, two on the East Glen, and one downstream of their confluence (Figure 3.8). On the West Glen, three sites (3-5, Figure 3.8) typically have perennial flow, however, site 4 has experienced streambed drying in recent years due to the appearance of sinkholes and was therefore considered as intermittent in the current investigation; two other sites (1-2) also have intermittent flow. On the East Glen one site (7, Figure 3.8) is intermittent and the other (6) is perennial but becomes ponded during periods of low flow. Downstream of the confluence the river is perennial and becomes navigable; flow variability in this widened, deepened stretch is very limited and this area was therefore not considered by the current investigation.

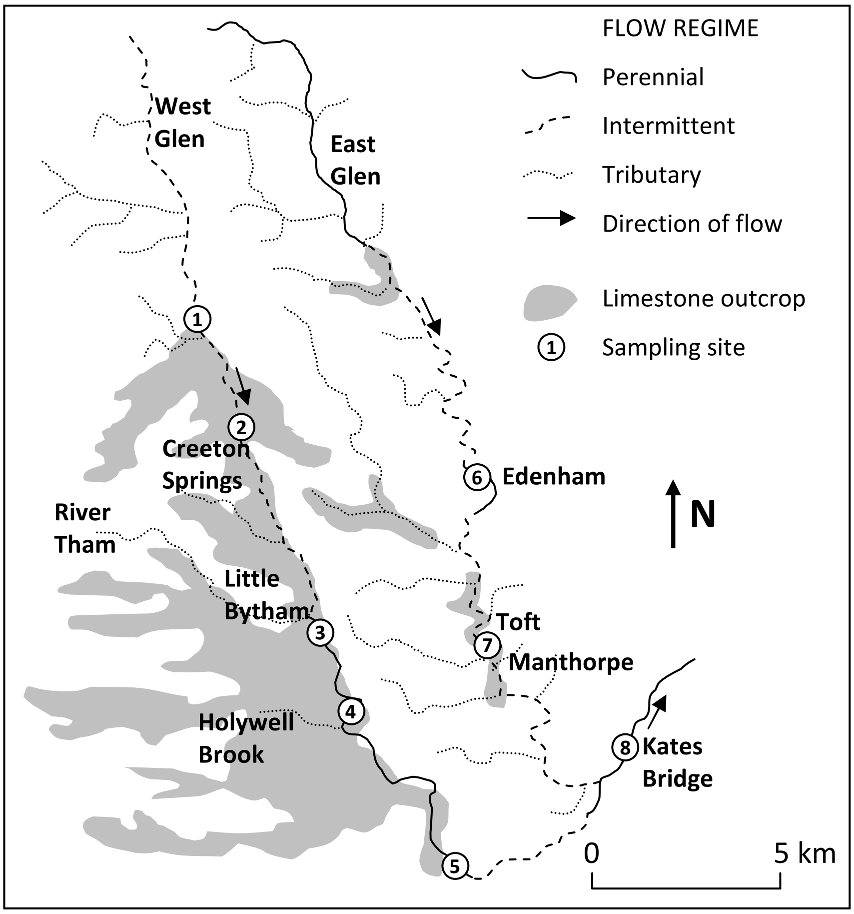


Figure 3.8: Location map of the River Glen, indicating sampling points.

3.5.2 Baseline survey sampling strategy

Baseline surveys of the benthic invertebrate community were conducted at the eight River Glen and nine River Lathkill sites in October 2007. Autumn sampling maximises the collection of the full complement of species present, since those insect taxa that are absent from the aquatic environment during the summer months have returned (Resh, 1979). Two complementary techniques, kick sampling and Surber sampling, were used. At each site, between three and five quantitative 30-second Surber samples were taken from riffle and run habitats. The number of samples taken reflected the size of the site, with care being taken to characterise the site thoroughly whilst avoiding unnecessary levels of disturbance. In addition, one kick sample was taken at each site for a more comprehensive characterisation of the invertebrate community, including those species present in non-riffle habitats. Kick sampling was undertaken for a period of between 1-3 minutes, again reflecting the size of the site and variety of habitats present. Rationale governing the choice of these techniques is provided in section 4.4.

3.5.3 Analysis of baseline survey data

Invertebrate community composition was analysed separately for each river. All quantitative community data (i.e. all Surber samples) were analysed using correspondence analysis (CA) in the program Canoco for Windows Version 4.54 (ter Braak & Šmilauer, 2006); preliminary analysis indicated that detrending distorted the ordination (Kenkel & Orlóci, 1986). Prior to analysis, data were log transformed (ln + 1) to reduce skewness in the data distribution caused by the dominance of the few most abundant taxa (ter Braak, 1995), and rare taxa were downweighted to reduce their influence on the overall pattern of community change.

3.5.4 Results of the baseline survey

CA ordination of the River Lathkill baseline survey data (Figure 3.9) indicated that most sites had distinct invertebrate communities, although overlap existed between two adjacent perennial sites (2 and 3) and between two adjacent intermittent sites (7 and 8). Samples were separated according to the site flow permanence regime along Axis 2 of the ordination, with intermittent and ephemeral sites plotting almost exclusively in negative quadrants and perennial sites predominantly in positive

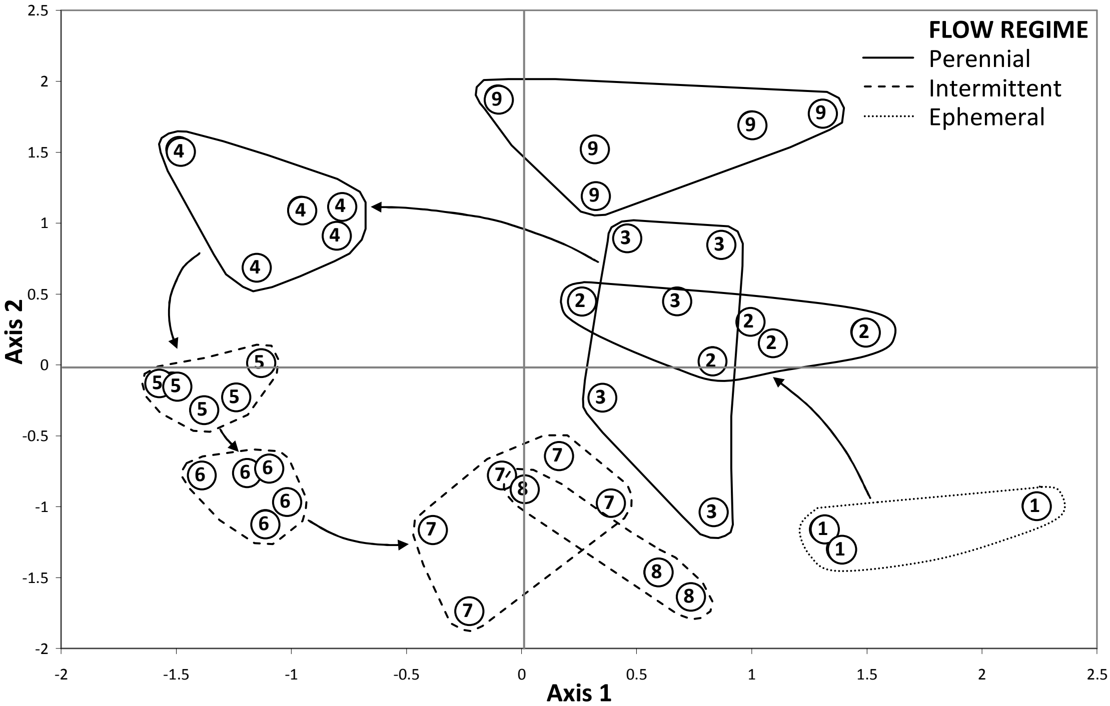


Figure 3.9: Correspondence analysis of Surber samples collected from the River Lathkill, indicating flow permanence regime. Numbers refer to sampling sites (see Figure 3.7). Arrows indicate relative positions of sites, from upstream (1) to downstream (8).

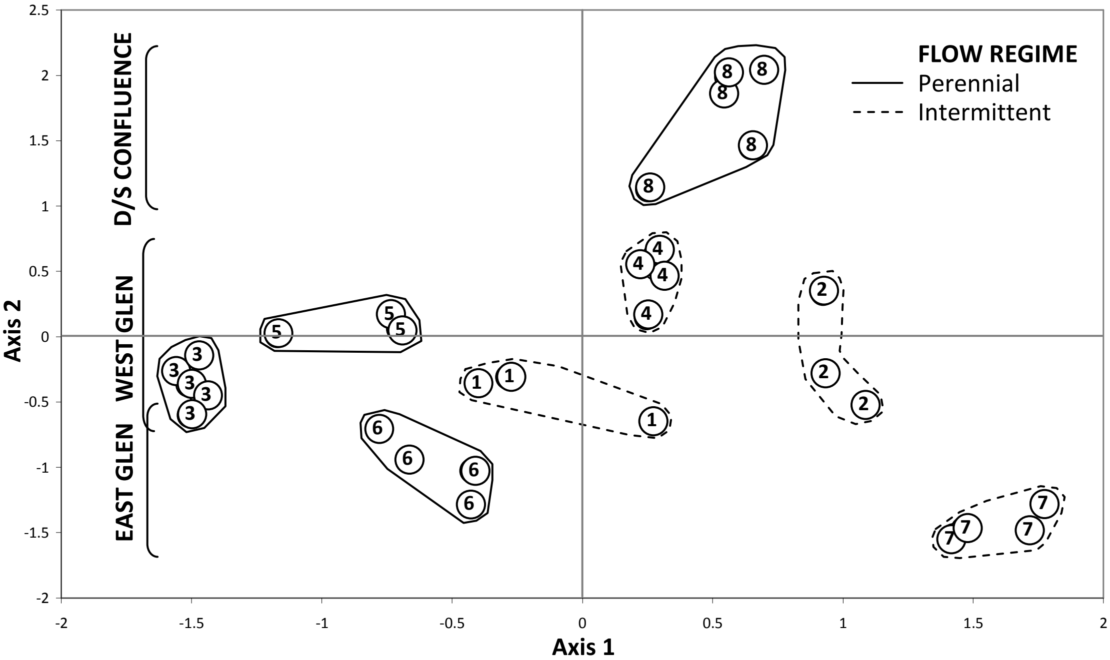


Figure 3.10: Correspondence analysis of baseline survey Surber samples collected from the Rivers West and East Glen, indicating flow permanence regime. Numbers indicate sampling sites (see Figure 3.8).

quadrants. In addition, the longitudinal distribution of sites 1-8 was observed as an anti-clockwise pattern. Site 9, the perennial head of the river, is located in a much larger and more hydrologically stable section of the river and was excluded from this pattern, with samples having particularly high Axis 2 scores (Figure 3.9).

CA of the River Glen baseline survey data demonstrated clustering of samples from individual sites, with little overlap between sites (Figure 3.10). Sites on both the East and West Glen tributaries were separated according to flow permanence regime on Axis 1, with intermittent sites plotting mainly in positive quadrants and perennial sites exclusively in negative quadrants (Figure 3.10). In addition, the West Glen samples were distinct from East Glen samples, the latter having lower scores on Axis 2. The single site located downstream of the East and West Glen confluence was the only perennial site to plot in a positive quadrant of Axis 1 and this site also had particularly high Axis 2 scores (Figure 3.10).

3.5.5 Selection of study sites

Sites were required on both rivers for the implementation of field experiments considering benthic and hyporheic invertebrate community responses to flow variability. A total of eight sites was considered as the maximum that could be investigated in sufficient detail in the time available. These eight sites were divided into four per river, with two sites with perennial flow and two with intermittent flow selected on each. CA identified overlaps in community composition between sites and the ordinations were therefore used to inform the selection of distinct sites. Observations made at each site were also considered to ensure exclusion of atypical sites, for example River Glen sites with evidence of damaging management practices (e.g. dredging) that could have unpredictable effects on instream communities were discounted. Where multiple sites appeared equally appropriate for investigation, sites were selected randomly to avoid the introduction of bias (Gordon *et al*., 2004).

On the River Lathkill, three sites were excluded based on on-site observations: site 1 (Figure 3.7), in the upper headwaters, was dominated by boulders and lacked a well-developed hyporheic zone; the hydrological variability of site 8 was compromised by a downstream weir; and site 9 was too heterogeneous to select a practical number of representative sampling points. Three of the remaining six sites were perennial: sites 2, 3 and 4. The CA ordination (Figure 3.9) indicated considerable overlap between sites 2 and 3, and site 2 was therefore excluded due to practical considerations (it is particularly remote), leaving sites 3 and 4 as the two perennial sites. Of the three remaining intermittent sites (5, 6 and 7), site 6 was discarded as sites 5 and 7 were more distinct from one another. Sites 3, 4, 5 and 7 will be referred to as 1, 2, 3 and 4 respectively from this point onwards (Figure 3.11; explanation of additional site 5 is provided below). Numerically, the communities at these sites were dominated primarily by *Gammarus pulex* (Amphipoda: Crustacea) and also by species of Chironomidae (Diptera). Taxonomically, insect groups including the Plecoptera, Trichoptera and Coleoptera species were particularly diverse. Many taxa were common to all sites, whilst temporary water specialists such *Helophorus brevipalpis* (Coleoptera) were restricted to intermittent sites and stenothermic taxa such as *Crenobia alpina* (Turbellaria: Planariidae) occurred predominantly at sites with perennial flow. Species lists are presented for the River Lathkill in Appendix 1.

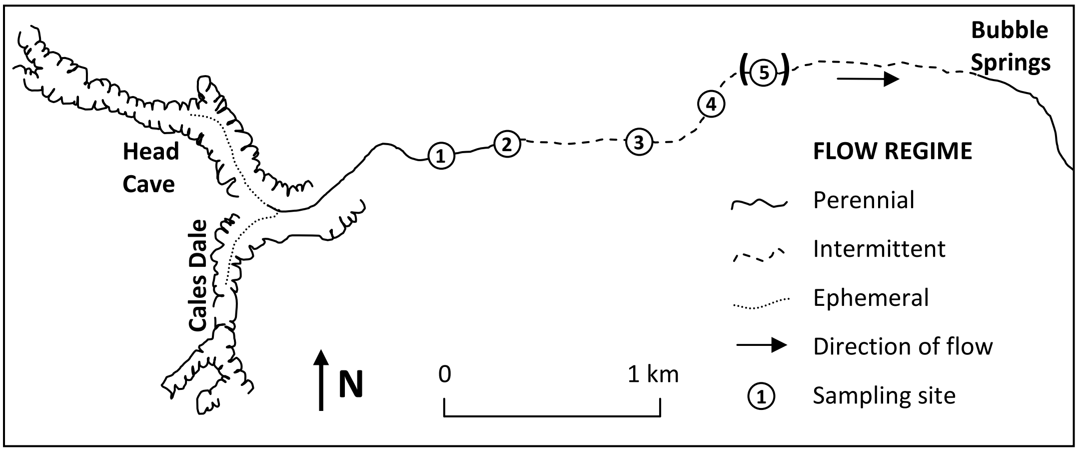


Figure 3.11: Selected sites for the investigation of benthic and hyporheic community responses to flow variability on the River Lathkill.

An additional site was selected on the River Lathkill for detailed examination of environmental changes in hyporheic habitat during streambed drying (although these additional environmental data were not ultimately of relevance to the current investigation). This site (5, Figure 3.11) was selected following consultation with Philip Bowler of Natural England and John Gunn of the University of Birmingham. Principal selection criteria were: i) a high probability of streambed drying occurring during the summer months; and ii) a sufficient depth of hyporheic sediments to install hyporheic monitoring equipment. To characterise the community response to any observed environmental variability, this site was added to the eight previously selected sites for characterisation of benthic-hyporheic interactions. A baseline survey was conducted at this site in April 2008, which confirmed that both the benthic and hyporheic invertebrate communities were sufficiently diverse and abundant to justify further investigation.

On the River Glen, site 8 (Figure 3.8) was excluded due to the decline in hydrological variability downstream of the East and West Glen confluence, and sites 1, 2 and 5 were too small to permit the collection of sufficient replicate samples without causing unacceptable levels of instream disturbance. The remaining four sites (3, 4, 6 and 7) comprised one perennial and one intermittent site on the West Glen and the same on the East Glen. CA ordination (Figure 3.10) confirmed that samples collected at these four sites formed distinct clusters, indicating their potential to provide good levels of within-site replication during the subsequent sampling programme. Sites 3, 4, 6 and 7 will be referred to as sites 1, 2, 3 and 4 respectively from here on in (Figure 3.12). The most abundant taxa at these sites were the Chironomidae and Oligochaeta, and *Gammarus pulex* was also very common. The Trichoptera and Gastropoda were taxonomically the most diverse groups, whilst Plecoptera were absent. Species lists for the River Glen baseline survey are provided in Appendix 2.

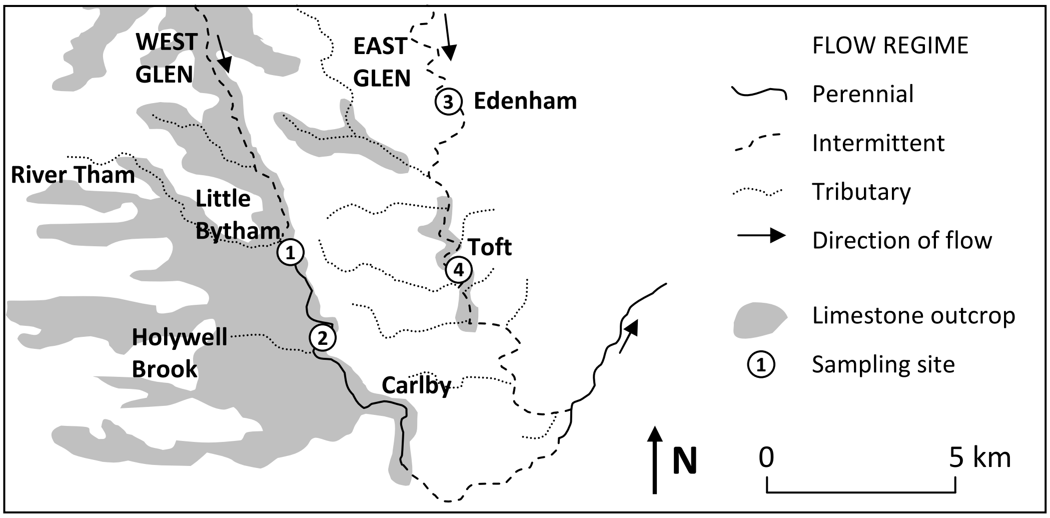


Figure 3.12: Selected sites (1-4) for the investigation of benthic and hyporheic community responses to flow variability on the River Glen.

Further preliminary surveys were conducted at the selected five River Lathkill and four River Glen sites in February-March 2008, to characterise both the benthic and the hyporheic invertebrate communities. These surveys were intended to coincide with conditions of ‘normal’ flow, i.e. no extreme hydrological events (spates, streambed drying) were known to have occurred in the weeks preceding sampling or during sampling. The benthos was re-sampled to complete the characterisation of the communities present, and at the same locations, hyporheic sampling was conducted to ensure that a well-developed hyporheos was present. Samples were collected from 20 cm into the hyporheic sediments, as this depth is typically inhabited by a diverse and abundant hyporheos compared with greater depths (Marchant, 1988; Adkins & Winterbourn, 1999). Hyporheic sampling techniques are described and justified in section 4.4.2. The results of the hyporheic survey (presented in Appendices 3 and 4) indicated that the hyporheos was sufficiently diverse and abundant at all sites to warrant further investigation; no changes to the selected eight sites were therefore deemed necessary.

3.6 Selection of sampling areas

At each site, all sampling points were situated in homogeneous habitat that represented the prevailing conditions in terms of substrate composition and surface hydrology (Table 3.2). Care was taken to avoid atypical areas, such as mid-channel submerged macrophyte beds, marginal reed beds and backwaters. Mid-channel topographic high points were also avoided, as these areas were likely to dry earlier than is typical, although relatively shallow marginal areas were selected where mid-channel conditions were too deep or fast-flowing to permit safe sampling. At each site, sampling points were positioned several metres apart to prevent sampling at one location disturbing adjacent sampling points. Plans of each site including sampling points are presented in Appendices 5 and 6.

Previous investigations of the Surber sampling technique (see section 4.4.1) have indicated that sampling effort has a profound impact on the recorded taxon richness of the invertebrate community (Li *et al*., 2001), with some studies suggesting a prohibitively high number of samples as necessary to fully represent some taxonomic groups (e.g. 20 samples per riffle for caddisfly larvae; Schmera & Eros, 2006). However, even attempts to characterise the complete species complement present within a large spatial area have recorded some levelling off of taxonomic richness after a relatively small number of samples. Li *et al*. (2001), for example, found that the rate at which new taxa were added to the species pool of first- to third-order streams began to slow after the fourth sample; similarly, Chiasson (2009) suggest four Surber samples as the minimum required to characterise taxon richness in Canadian streams of contrasting water quality. Therefore, five sampling points were initially selected to characterise the invertebrate community at each site. However, time constraints necessitated the downward revision of this number, and preliminary analysis confirmed that the fifth sample did not significantly improve estimations of either invertebrate density or taxonomic richness (data not presented). Therefore, four sampling points were retained at each site.

Table 3.2: Dominant habitat features at sampling points selected for the investigation of benthic and hyporheic community responses to flow variability. Locations of each site are provided in Figures 3.11 and 3.12

RIVER	Site no.	Hydrological habitat type	Surface substrata	Vegetation
Lathkill	1	Riffle-run	Cobbles and gravel	Marginal reeds and mint
	2	Riffle	Gravel, cobbles and boulders	Liverwort, moss
	3	Riffle-run	Cobbles and boulders	Liverwort, moss Marginal reeds and mint
	4	Run-glide	Sand, gravel and cobbles	Marginal reeds
	5	Glide	Gravel and sand	Sparse
Glen	1	Riffle-run	Patchy: Gravel, clay & cobbles	Mid-channel *Ranunculus* sp.
	2	Run	Gravel	Mid-channel *Ranunculus* sp., Marginal reeds
	3	Glide	Gravel, silt covered	Patchy *Cladophora* sp.
	4	Riffle-run	Gravel and cobbles	*Cladophora* sp. Marginal reeds

3.7 Summary

This chapter has outlined the process of site selection, from the choice of river type to the positioning of sampling points within the sites selected on each river. As a result, 36 sampling points at nine sites (i.e. four sampling points per site) across two rivers have been selected for detailed investigation of invertebrate community responses to hydrological variability.

4. Methodological Approaches and Sampling Techniques

4.1 Introduction

This chapter examines the range of methodological approaches adopted to achieve the thesis aims (Table 4.1). The techniques used to sample invertebrate communities and characterise the physical environment are described in detail. The advantages and limitations of the approaches used are evaluated and the selection of each technique justified. The biotic indices, statistical approaches and ordination techniques used to analyse biological and environmental data are also discussed, as are the analytical approaches used to examine relationships between the biota and their environment.

4.2 General methodological approach

To address questions concerning community responses to environmental variability, a choice must be made between laboratory experiment, field experiment and natural experiment approaches (Diamond, 1986). Although laboratory experiments allow the greatest control of environmental variables, their ability to represent complex ecosystems is severely restricted. At the other extreme, natural experiments clearly have the advantage of maximising realism, however, this is gained at the expense of control over the environmental variables under consideration (Diamond, 1986; Blackburn, 2004). Intermediate between these approaches, field experiments also record responses of natural, complex instream communities, but allow manipulation of environmental conditions. However, field experiments are typically limited in spatial scale, may produce misleading data due to variation in unmeasured and uncontrolled variables, and often fail to consider the secondary effects of changes in the variables of interest (Diamond, 1986; James *et al.*, 2008).

In the current investigation of community responses to hydrological variability (aims 1 and 2, Table 4.1), numerous secondary environmental variables that change in response to hydrological variability were of potential importance in determining instream community composition (Dewson *et al*., 2007a). In addition, the flow regime is to some extent temporally predictable, depending on climatic and geological controls (Monk *et al*., 2006), and hydrological variability can also be spatially predictable as a result of variation in the underlying geology (e.g. Maddock *et al*., 1995). Therefore, natural trajectory experiments (NTEs) ('comparisons of the same community at various times before, during, and after a witnessed perturbation by nature'; Diamond, 1986, p. 4) were selected as the most appropriate approach to investigate benthic and hyporheic community responses to flow variability, and were sited and implemented to correspond with the environmental conditions of interest.

4.3 Sampling strategy

NTEs to characterise benthic and hyporheic invertebrate community responses to flow variability were conducted at five River Lathkill and four River Glen sites between May and September 2008, with the exception of Lathkill site 5 (Figure 3.11), where water was too deep to permit sampling until June. Lathkill site 5 was also sampled between May and September 2009, to provide data for inter-annual comparisons. Conducting the investigation during the summer months maximised the probability of flow recession, low flows and streambed drying occurring during the study period, and the sampling programme was terminated in September following a spate on the River Lathkill (Figure 5.3) and an increase in discharge on the River Glen (Figure 6.2). Sampling was conducted at monthly intervals; more frequent sampling was not practical as samples needed to be taken from the same locations on each occasion, and it was therefore necessary to leave time for invertebrate recolonisation.

At each of the nine sampling sites, four samples were taken to characterise the benthic community, and below each benthic sampling position, samples of the hyporheic community were obtained from depths of 10 cm, 20 cm and 30 cm (Figure 4.1; also see section 3.6). Thus, one benthic and three hyporheic invertebrate samples were collected from 36 sampling points each month for five months in 2008, and from four sampling points each month for five months in 2009 (Table 4.1). To minimise disturbance of each sampling area prior to sample acquisition, benthic samples were collected before hyporheic samples, and sampling points were visited from downstream to upstream.

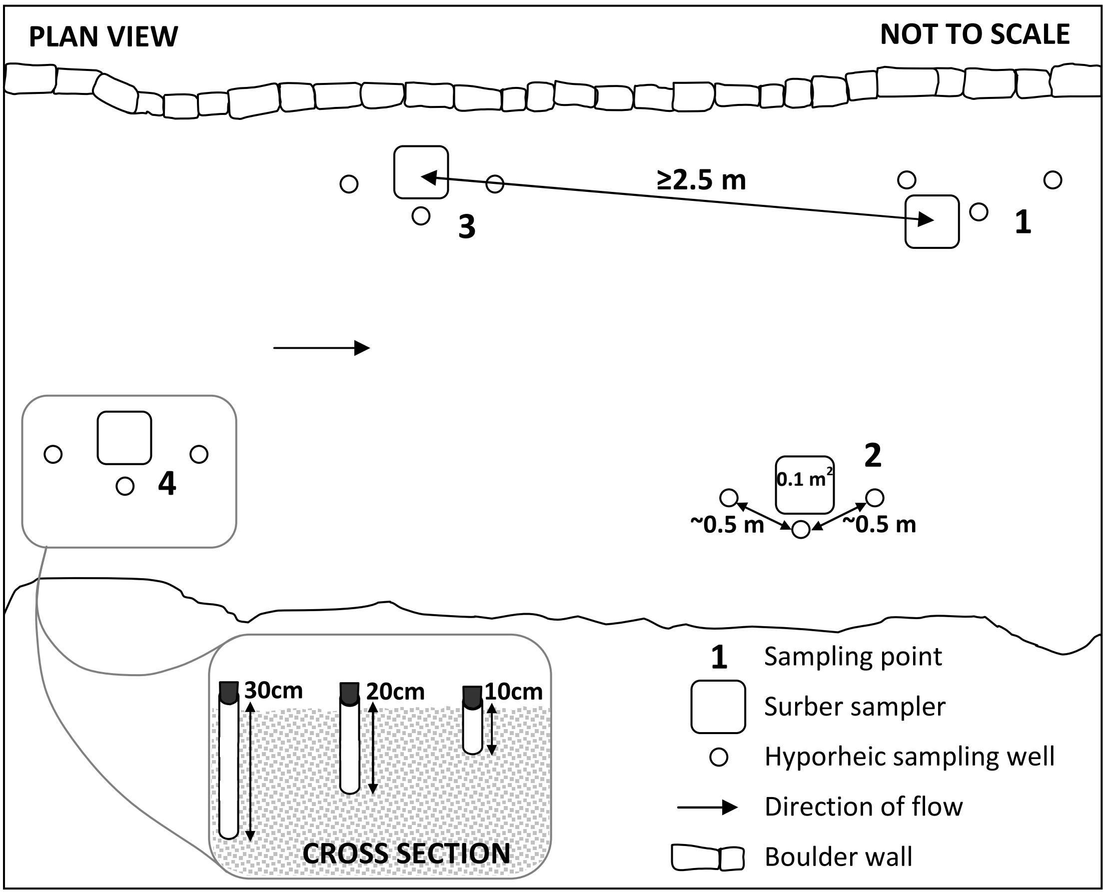


Figure 4.1: Sampling locations at a typical study site for the investigation of benthic and hyporheic community responses to flow variability.

Table 4.1: Summary of the methodological approaches, sampling strategy and analytical approaches used to achieve the thesis aims

THESIS AIM	CHAPTER	APPROACH	DURATION	LOCATION	TOTAL SAMPLES	DATA TYPE*	ANALYTICAL APPROACH**
1. Identify hydrological and hydrologically mediated benthic invertebrate stressors **2.** Examine benthic invertebrate response to identified stressors	5	Natural trajectory experiments (NTEs)	May–Sept 2008 (+ Lathkill site 5 May–Sept 2009)	Lathkill, 5 sites	400 (¼ BI; ¾ HI)	1. ENV, DIS 2.BI, HI, SED	1. FDA, RM ANOVA, PCA 2. RM ANOVA, DCA, CCA, correlation
	6			Glen, 4 sites	320 (¼ BI; ¾ HI)		
3. Develop conceptual models relating benthic use of hyporheic zone to spatio-temporal environmental variability	7	Synthesis of other objectives	N/A	All	All	BI, HI, ENV, SED	Synthesis of analysed data and previous research

*BI = benthic invertebrates; HI = hyporheic invertebrates; SED = sediments; ENV = site-specific environmental data (including hydrological measurements); DIS = discharge.
** FDA = flow duration analysis; RM ANOVA = repeated measures analysis of variance; PCA = principal components analysis; DCA = detrended correspondence analysis; CCA = canonical correspondence analysis

4.4 Invertebrate sampling, processing and identification

Ecological investigations should be conducted using invertebrate sampling techniques that promote accurate representation of the communities under scrutiny (Cao *et al*., 2002). In addition, in the current investigation, the following criteria were particular requirements of selected invertebrate sampling techniques:

1. Quantitative samples were required, to facilitate observation of spatial and temporal changes in community composition and abundance. An exception to this was in the collection of baseline survey data, for which it was appropriate to supplement a quantitative approach with semi-quantitative data to improve overall community characterisation.
2. A sampling procedure was required which did not cause lasting damage to the invertebrate community or physical environment, as this would have invalidated repeated sampling at the same location.
3. Whilst different techniques were required to characterise the various invertebrate communities (i.e. benthic and hyporheic) and to sample under different hydrological conditions (i.e. during periods of streamflow and following possible streambed drying), these needed to be comparable.
4. Sampling techniques needed to be operable in conditions spanning the full spatiotemporal range of depth and flow conditions expected during the investigation.
5. All techniques needed to allow relatively rapid collection of invertebrate samples, to facilitate acquisition of sufficient replicates.
6. Sampling equipment needed to be easily transportable by two people, as some sampling locations were remote.

In both benthic and hyporheic habitats, all standard sampling techniques have certain limitations (Storey *et al*., 1991; Fraser and Williams, 1997), although widely accepted procedures now exist for sampling benthic communities and are evolving for the hyporheic fauna. However, invertebrate collection in unusual streambed environments may necessitate the development of specific techniques; this was the case following potential streambed drying at intermittent alluvial sites on both rivers. A description and critical evaluation of all selected standard techniques and supplementary techniques developed during the investigation follows in sections 4.4.1 to 4.4.3.

4.4.1 Benthic invertebrate sampling techniques

Two procedures were used to sample benthic invertebrates in flowing water, Surber sampling and kick sampling.

The Surber sampler is essentially a 1 mm mesh net attached to a 0.1 m^2 quadrat (Surber, 1970). The Surber frame is placed on the substrate surface with the net opening facing into the current, and the substrate within the frame disturbed manually to a depth of 5-10 cm (depending on substrate) for 30 seconds (this duration being demonstrated as sufficient to dislodge and capture the majority of invertebrates present; Hughes, 1978). Surber sampling has the principal advantage of being fully quantitative, is simple and requires minimal specialist equipment. In addition, Surber sampling causes little disturbance to the surrounding area, and invertebrate recolonisation of the sampled area is completed within 30 days (Matthaei *et al*., 1996); repeated sampling can therefore be performed during a temporal sequence. This technique is in widespread use in hydro-ecological research projects that require quantification of the benthic invertebrate community (e.g. Matthaei *et al*., 1997; Dolédec *et al*., 2007), thus facilitating comparison with previous research. Limitations of Surber sampling include potential underestimation of taxon richness and invertebrate abundance (Brooks, 1994; Adkins and Winterbourn, 1999), whilst the small sampling area makes the technique susceptible to recording instream patchiness, particularly in heterogeneous environments (Hornig and Pollard, 1978). Representative samples are therefore more likely to be collected if sampling points are positioned carefully, at the expense of randomization (Gordon *et al*., 2004). In the current investigation, Surber samples were taken during baseline surveys, and were routinely used at all sampling sites to quantify changes in the benthic community in response to hydrological variability (Chapters 5 and 6).

The kick sampling technique also requires only a single piece of equipment, a pond or hand net, which consists of a net bag (1 mm mesh, 230 x 255 mm frame, 275 mm bag depth) attached to a 1.5 m handle (Furse *et al*., 1981). To take a sample, the net frame is positioned on the streambed with the bag opening facing into the current. The substrate directly upstream of the net opening is disturbed using the feet, with the current carrying the dislodged invertebrates into the net. The operator moves around the site for a pre-defined time period (typically 1-3 minutes), spending a proportional amount of time sampling in each habitat present (FBA, 2009). This technique is semi-quantitative if conducted for a pre-defined length of time, and has the advantage of covering a relatively large area of the streambed, including the full range of habitats present. It therefore samples a relatively large proportion of the invertebrate taxa present and is more likely to capture rare taxa than Surber sampling (Chiasson, 2009). However, cryptic and closely adherent taxa, which are not easily dislodged from the substrate, maybe underrepresented (Storey *et al*., 1991), and kick sampling should therefore be supplemented by manual inspection of large clasts. Kick samples were only used during baseline surveys (section 3.5), to improve the characterisation of the benthic invertebrate community provided by Surber sampling.

4.4.2 Hyporheic invertebrate sampling techniques

As research in hyporheic ecology has gathered pace in the last few decades (Boulton *et al*., 1998), four categories of sampler designed to collect hyporheic invertebrates have emerged: freeze-coring, non-frozen sediment coring, installation of artificial substrates, and various pump sampling procedures (Fraser and Williams, 1997; Hunt and Stanley, 2000). The inaccessibility of the hyporheic habitat means that all techniques have known limitations (Palmer, 1993), and no consensus has been reached regarding the technique that provides the most representative samples (Hunt and Stanley, 2000). The decision to use manual bilge-pump sampling in the current investigation was therefore made based partly due to logistic issues.

Pump sampling, as outlined by Boulton *et al*. (1992) involves the extraction of a known volume of water from a pre-installed sampling well using a manual bilge pump. Limitations of this method include the difficulty in delimiting the area of sediment sampled (Soulsby *et al*., 2009), a bias favouring the collection of smaller, less tenacious invertebrates (Fraser and Williams, 1997), and variable efficacy depending on the hydraulic conductivity of the sediments (Scarsbrook and Halliday, 2002). However, this technique has several advantages that made it an appropriate choice for the characterisation of the hyporheic invertebrate community response to hydrological variability in the current investigation. Crucially, pump sampling causes minimal disturbance to the sediments and therefore allows repeated sample collection from the same location during a temporal sequence. Compared to other techniques, additional advantages of pump sampling are that it requires little specialist equipment, is quantitative, and is relatively quick and easy to conduct (Stubbington *et al*., 2009b). In addition, several studies have demonstrated the ability of pump sampling to effectively identify changes in hyporheic community composition in response to hydrological variability (Hancock, 2006; Datry *et al*., 2007). In conjunction with Surber samples of the benthic community, pump sampling was routinely used at all study sites to characterise changes in the hyporheic community in response to variation in surface flow (Chapters 5 and 6).

In preparation for the collection of hyporheic pump samples, three polyvinylchloride (PVC) pipes (19 mm internal diameter) were inserted at each sampling point to depths of 10 cm, 20 cm and 30 cm respectively by placing a pipe onto the end of a stainless steel T-bar and driving this into the sediments to the required depth using a lump hammer. These pipes were installed prior to the start of the sampling programme and functioned as hyporheic sampling wells for the duration of the study; lost wells were replaced at the same location as required. Wells were placed ~50 cm apart to minimise the effects of sampling in one well on the area of sediments sampled by adjacent wells.

On each sampling occasion, 6 L of hyporheic water were pumped from the base of each well in three 2 L aliquots. Hunt and Stanley (2000) demonstrated that sample volume was negatively correlated with estimates of invertebrate abundance, but positively related to the recorded taxon richness. Therefore, the volume selected is not of great importance providing that this volume remains constant on all sampling occasions. The volume used in the current investigation was selected to facilitate comparison with previous research (Boulton *et al.*, 1992; Boulton *et al.*, 2004).

4.4.3 Invertebrate sampling following streambed drying

An additional procedure to sample invertebrates from dry alluvial sediments was required following potential habitat contraction at any site, and following potential streambed drying at intermittent sites. Most previous research considering invertebrate community composition in relation to flow permanence has ceased sampling following the loss of surface water (e.g. Extence, 1981; Meyer and Meyer, 2000) and standard sampling procedures have therefore not been established. A simple method involving excavation of sediment and invertebrates to a depth of 5 cm was used (i.e. a modified Chappuis-Karaman method); this facilitated comparison with Surber samples. Due to the large volume of sediment collected using this method, an area of 0.05 m^2 (i.e. half the area sampled with a Surber net) was sampled to reduce habitat disturbance whilst still allowing comparison with samples taken in submerged conditions. A similar procedure described by del Rosario and Resh (2000) was successfully used to compare benthic community composition in an intermittent stream before and after the loss of surface water. In the present study, this sampling technique was only required at two Lathkill sampling points in August and issues related to habitat disturbance were therefore minimised; September sampling was conducted in undisturbed sediments in the immediate vicinity. This technique could have been adapted to sample dry hyporheic sediments, but the hyporheic zone remained submerged throughout the study. However, following the loss of surface water in some areas of the River Lathkill, a decline in hydraulic conductivity impeded pumping of hyporheic water and thus the acquisition of invertebrate samples. In the few instances when this occurred, a continuous stream of filtered river water was poured into the hyporheic sampling well during pumping, to act as a medium in which hyporheic invertebrates could be transported to the surface; hyporheic water physicochemistry could not be analysed in these circumstances.

4.4.4 Processing of invertebrate samples

All invertebrate samples were preserved using a 4 % formaldehyde solution and refrigerated at 4 °C following collection. Sample processing was subsequently conducted after an interval of between one day and several months. Each Surber or kick sample was emptied into a 250 µm sieve and rinsed thoroughly to remove fine material and traces of formaldehyde. Clean samples were transferred to a white, flat-bottomed tray on which quarters had been delineated, covered with water and the material spread out evenly. Each quarter of a sample was examined in turn and all invertebrates removed. If a taxon was particularly abundant, a sub-sample was taken by removing a known fraction (i.e. ⅛, ¼, ½, or ¾) of the sample. Preliminary tests conducted for several common taxa indicated that a minimum of 100 individuals of a taxon should be removed to obtain an accurate estimate of the total population. Hyporheic pump samples were also processed following this procedure, but invertebrates occurred at low densities and subsampling was not necessary. All observed invertebrates were removed and stored in 70% IMS prior to identification.

4.4.5 Identification of invertebrate taxa

All invertebrates were identified under a dissection microscope using standard UK taxonomic keys. Wherever possible, invertebrates were identified to species level to facilitate the identification of relationships between community composition and environmental conditions (Lenat and Resh, 2001). However, some taxa were left at higher levels of taxonomic resolution due to time constraints and the available expertise. Thus, *Succinea* (Gastropoda), *Baetis* (Ephemeroptera), early instar *Nemoura* and *Leuctra* (Plecoptera), adult *Oulimnius,* larval *Helophorus*, *Hydroporus, Hydraena* and *Riolus* (Coleoptera) and larval *Dicranota* and *Dixa* (Diptera) were identified to genus; Sphaeriidae (Bivalvia), larval Curculionidae, Dytiscidae and Scirtidae (Coleoptera), Corixidae (Hemiptera) nymphs and some Diptera (Ceratopogonidae, Chironomidae, Empididae, Muscidae, Psychodidae, Stratiomyidae, Simuliidae and Tipulidae (excluding *Dicranota* spp.)) were identified to family level; and Cladocera, Cyclopoida and Harpacticoida (Copepoda), Ostracoda, Nematoda, Oligochaeta and Hydracarina were left at the group level.

4.5 Sampling and analysis of environmental parameters

4.5.1 Rationale

Characterisation of hydrological parameters was clearly of prime importance, and measurements of water depth, wetted width and mean (0.6 x depth) flow velocity in the surface channel were supplemented by determination of vertical hydraulic gradient (VHG) at several individual sampling points. Changes in water physicochemistry that can accompany variation in flow also required quantification, and this was achieved primarily by taking spot measurements *in situ* (temperature, pH, conductivity and dissolved oxygen (DO)); these are standard variables that provide a general description of the conditions experienced by instream communities. In addition, water samples were collected for subsequent laboratory analysis of nitrate, phosphate, fine sediment and particulate organic carbon (POC) concentrations. Nutrient concentrations are also a standard measure of water quality, and nutrients, organic matter and fine sediment concentrations may vary in response to variation in surface flow (Caruso, 2002; Dewson *et al*., 2007a).

Although reliance on spot measurements can obscure temporal variability and extreme values (e.g. Jarvie *et al*., 2001; Soulsby *et al*., 2009), continuous monitoring was not generally possible in the current investigation due to the prohibitively high cost of installing monitoring equipment at multiple locations. Continuous monitoring of water temperature and sediment moisture content was, however, conducted in the benthic and hyporheic sediments at one site (Lathkill site 5) and these data were available to supplement spot measurements.

4.5.2 Collection of environmental data

Hydrological variables

Water depth and mean flow velocity (at 0.6 x depth) were measured using an ADS SENSA-RC2 flow meter (ADS Environmental Services, Huntsville, USA). Depth was subsequently used to estimate wetted width from multiple cross-sectional profiles of the river at each site (Appendices 7 and 8). Vertical hydraulic gradient (VHG) was measured using mini-piezometers (Lee and Cherry, 1979) at each sampling point at all sites on the River Glen and two sites on the River Lathkill; the remaining three sites on the Lathkill are clearly visible to visitors to the Dale and as such visual disturbance of these areas was undesirable. Mini-piezometers consisted of two 65 cm lengths of PVC pipe (19 mm internal diameter), one intact and the other perforated with four rows of 2 mm diameter holes at 2 cm intervals along the length of the pipe. Mini-piezometers were inserted into the river bed to a depth of 30 cm, using the same procedure as used for the hyporheic sampling wells (see Section 4.4.2), left to equilibrate for ≥2 weeks before use, and remained *in situ* throughout the investigation. Hydraulic head was measured by inserting an electrical dipstick into each of the two mini-piezometers and comparing the water levels recorded.

Submerged habitat availability

Cross-sectional profiles of each site (see section 4.7) and observations of instream conditions were used to determine the maximum extent of submerged benthic habitat recorded during the study. One cross-section was considered representative of the prevailing conditions at the site for Lathkill sites 1, 2, 3 and 5 and Glen sites 1, 2 and 4, whilst two cross-sections were used at Lathkill site 4 and Glen site 3 due to intra-site variability in channel topography. Monthly water depth data were then applied to each cross-sectional profile to determine submerged benthic habitat available in each month as a percentage of the maximum recorded.

Water physicochemistry

The temperature (°C), pH, conductivity (µS cm^{-1}), and DO concentration (mg L^{-1} and % saturation) of surface water and water pumped from each hyporheic depth were determined *in situ* using standard instrumentation (Hanna Instruments, Leighton Buzzard). For hyporheic water samples, measurements were taken in the second of the three 2 L samples drawn from the sampling well. Although this water was drawn from an undefined volume of sediment, possible surface water contamination of the first aliquot is unlikely to have affected this second aliquot, due to the pressure head surrounding the base of the sampling well causing water to be drawn preferentially from below this level. Care was taken to ensure all probes had sufficient time to equilibrate prior to readings being taken.

Samples of surface water and hyporheic water from each depth were collected at one point per site. These samples were retained in acid-washed (2 % nitric acid solution) 250 ml bottles from which air was excluded, placed in black bags, transported to the laboratory and refrigerated at 4 ^{o}C prior to subsequent determination of nitrate, phosphate, fine sediment and POC concentrations. These samples were taken from the same sampling points each month. In addition, following completion of the sampling programme, surface and hyporheic water samples were collected from all four sampling points at two randomly selected sites and analysed to determine if single samples provided an accurate representation of the prevailing conditions at a site.

4.5.3 Laboratory procedures

Analysis of water samples to determine nitrate, phosphate, fine sediment and POC was performed within 24 hours of sample collection. Samples were kept refrigerated at 4 °C in darkness until processing. Nitrate and phosphate concentrations were determined using standard spectrophotometry reagents (Palintest Ltd, Gateshead, UK). POC and fine sediment concentrations were determined by hand-pumping the water sample through a Whatman glass fibre filter (GF/F, particle retention 0.7 µm; Whatman Plc, Maidstone, UK) that had previously been combusted for 2 hrs at 550°C and weighed. Sufficient water was filtered to discolour the filter paper, then discoloured papers were oven dried for 17 hrs at 105 °C, weighed, combusted for 2 hrs at 550 °C and reweighed.

4.6 Characterisation of alluvial sediments

4.6.1 Rationale

As the habitat in which invertebrates live, the nature of sediments play an important role in determining community composition (Olsen and Townsend, 2003; Larsen *et al*., 2009), community responses to hydrological variability (Dole-Olivier *et al*., 1997; Effenberger *et al*., 2008), and invertebrate survival following streambed drying (Barko and Smart, 1986; Clinton *et al*., 1996). It was therefore important to characterise sediments using techniques that maximised representivity. The hyporheic sediments can be heterogeneous over small areas (Salehin *et al*., 2004) and therefore representivity could only be ensured by collecting samples from invertebrate sampling locations. Sediment sampling in alluvial deposits can cause considerable localised habitat disturbance, and characterisation of the sediments could therefore only be undertaken once at each site, following the completion of the invertebrate sampling programme. Temporal variation in substrate composition could therefore not be examined in this investigation.

Quantification of the proportion of fine sediment was of particular importance for alluvial sediments, as this has a particular influence on hyporheic community composition (Richards and Bacon, 1994; Olsen and Townsend, 2003; Weigelhofer and Waringer, 2003). Whilst the definition of 'fine sediment' in previous studies is very variable (i.e. 63 µm – 1 mm, Olsen and Townsend, 2003; 150-850 µm, Richards and Bacon, 1994; <2 mm, Weigelhofer and Waringer (2003)), all refer to the importance of fine sediment which is coarse enough to be analysed using standard sieving techniques. Such techniques were therefore considered sufficient for describing the grain size distribution in the current investigation.

4.6.2 Sediment sampling techniques

Methods of obtaining sediment samples in submerged conditions fall into five main categories: shovel-type samplers, McNeil samplers, barrel samplers, freeze-core samplers and hybrid pipe freeze-core samplers (Bunte and Abt, 2001). Of these, shovel methods were discounted as they may severely underestimate the amount of fine sediments (Schuett-Hames *et al*., 1996), which are particularly important in determining the composition of the hyporheos (Richards and Bacon, 1994); barrel samplers were also not suitable as they sample only the top few centimetres of sediments. Freeze-coring and hybrid pipe freeze-coring were considered, as they have the advantage of obtaining stratified samples. However, these techniques were ultimately discounted due to logistic issues (i.e. transport of equipment to remote sites and equipment availability) and concerns over site disturbance; in addition, freeze-coring techniques have several disadvantages, including poor characterisation of the surface sediments and an unrepresentative dominance of large clasts (Kondolf *et al*., 2008).

The McNeil sampler (Figure 4.2) was therefore selected as the most appropriate sediment sampling device to characterise the sediments at each sampling point. This device was designed to collect fine sediments (McNeil and Ahnell, 1964), and some studies have indicated that it provides a more accurate representation of the grain-size distribution than other sampling techniques, including freeze-coring (Young *et al*., 1991). The principal disadvantage of McNeil sampling is that bulk, non-stratified samples are obtained; nonetheless, it can facilitate inter-site comparisons in ecological studies (Curry and MacNeill, 2004).

The McNeil sampler used comprised a small inner pipe (internal diameter 15 cm) which extended through the base of a larger outer cylinder (internal diameter 35 cm) to a depth of 25 cm (Figure 4.2). The top edge of the inner pipe also extended upwards into the interior of the large cylinder, creating a sediment storage area within the device. The serrated bottom edge of the small pipe was inserted into the sediments using a twisting motion to the maximum depth achievable, potentially 25 cm. Sediment within the small pipe was manually excavated into the surrounding storage area. Samples were collected at all sites and, whenever possible, at all four sampling points; sampling difficulties reduced the number of samples taken to three at several sites and to two at Lathkill site 3.

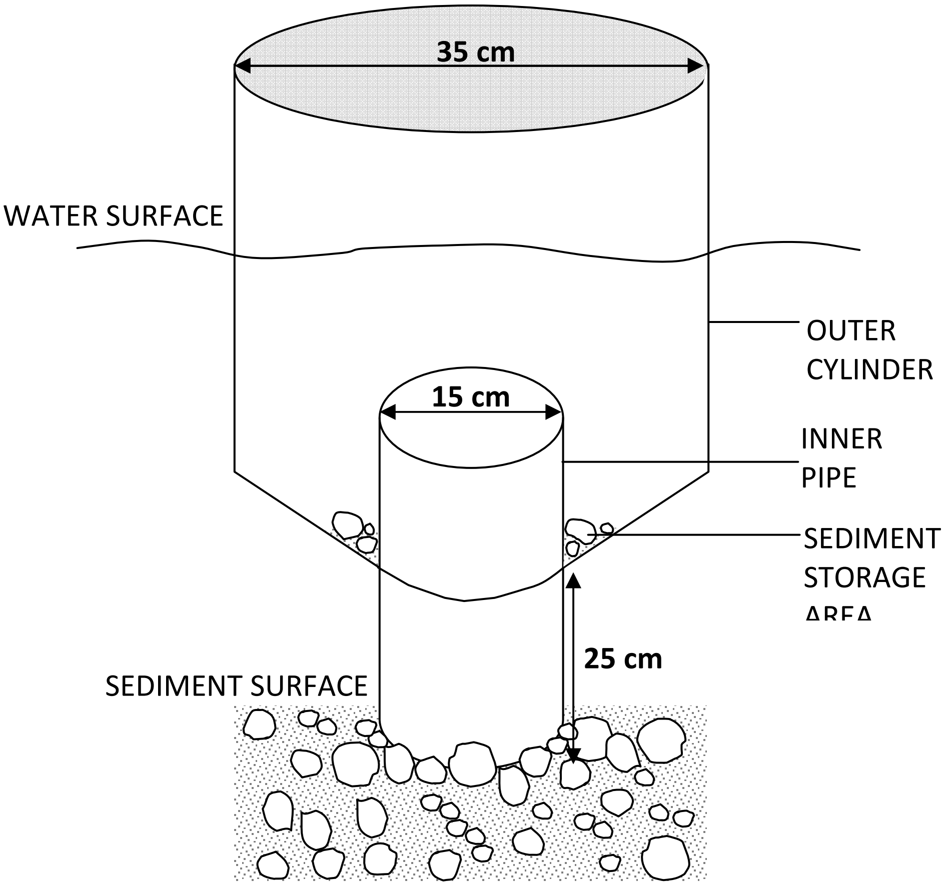


Figure 4.2: The McNeil sediment sampler

4.6.3 Laboratory analysis

All McNeil sediment samples were oven dried at 105 °C to ensure the release of all moisture associated with the sediment particles, including hygroscopic water (Mudroch and Bourbonniere, 1994). Samples were weighed every 12 hours and drying continued until sample weight remained constant. Oven-drying can result in aggregation of sediments when fine-grained material is present (Folk, 1980); however, it was necessary to ensure that samples were completely dry prior to determination of the grain-size distribution so that accurate weights were recorded. When necessary, dried samples were gently disaggregated by hand using a porcelain pestle and mortar, with care taken not to apply undue force that could result in comminution (Mudroch and Bourbonniere, 1994). Disaggregated sediments were then dry-sieved through a sieve nest (8, 4, 2, and 1 mm, 500, 250, 125 and 63 µm) using a sieve shaker (Endecotts Ltd, London, UK) for five minutes. Each size fraction was then weighed. To reduce the influence of atypically large particles on the calculated percentage of fine sediment, particles with an *a*-axis >75 mm were not weighed as part of the >8 mm size fraction. A truncation size of 75 mm was chosen following Rice (1995), as it is half the diameter of the small inner sampling pipe. Although this definition was somewhat arbitrary, adopting a consistent approach allowed subsequent comparison between samples (Rice, 1995).

4.7 Surveying of study sites

Following completion of the sampling programme, surveys of general instream habitat conditions were undertaken at each site, including ~50 m upstream and downstream of the study area. Measurements of channel width were taken at regular (<2 m) intervals and observations made regarding surrounding land use, riparian and instream vegetation, substrate composition, nature of the banks and shading, as well as other features of interest (e.g. flow deflectors, woody debris, anthropogenic debris). These observations and numerous photographs were used to create plan views of each site; these are provided in Appendices 5 and 6.

Standard tacheometry procedures employing a surveyor’s level were used to generate multiple cross-sectional profiles of the channel and banks at each site. This was undertaken to characterise variation in bed morphology and visualise the nature of the streambed and banks. In addition, one longitudinal profile of ~100 m was measured determine the slope of the channel bed. Results of the cross-sectional surveys are provided in Appendices 7 and 8.

4.8 Supplementary environmental data sources

4.8.1 Hydrological and meteorological data

Additional data were provided by various external organisations. For the Lathkill, continuous discharge data were obtained for the period preceding and including the study (1st April to 30th September 2008) from Prof. John Gunn (Limestone Research

Group, University of Birmingham) for Psalm Pool, ~1 km downstream of the study area, where discharge is augmented by groundwater springs (Figure 5.2). Mean hourly air temperature and rainfall measurements for the catchment were acquired for the same period from an automated weather station located at Wardlow Hay Cop, 8 km to the north (SK178739; also provided by John Gunn; Figure 5.1; Figure 5.2). This local air temperature data were supplemented by 2003-2007 monthly mean data, firstly from a Met Office station 44 km south-west of the Lathkill (Met Office, 2009a) and also from an independently-run station at Buxton, 14 km to the north-west (Hilton, 2009; Table 5.1). Long-term (1991-2000) average rainfall data for the Lathkill area were acquired for a Met Office land surface observation station located at Monyash, 4 km to the west of the river (BADC, 2009; Table 5.2).

For the River Glen, continuous discharge data were provided for the study period by the Environment Agency's Anglian Region office for each tributary (Figure 6.2). East Glen data are from Manthorpe, <1 km downstream of site 4; two tributaries join the river between site 4 and the gauging station, but these gains are offset by transmission losses through the streambed, resulting in a comparable flow regime (Figure 3.6; C. Extence, pers. comm.). Discharge data for the West Glen are from a site located <500 m upstream of site 1, with no tributaries or abstractions altering discharge between the two locations. Available air temperature data for the study period comprised 12-hourly minimum and maximum values from Monks Wood, Cambridgeshire (TL200801, 40 km to the south) and weekly means for MORECS (Meteorological Office Rainfall and Evapotranspiration Calculation System) square 118, which includes the Glen catchment in its south-western corner (NERC, 2009; Table 6.1; Figure 6.1). Long-term (1971-2000) average air temperature data were also available from Waddington, 40 km to the north at a similar elevation (Met Office 2009c; Table 6.2). Long-term (1980-2008) rainfall data for the Glen region were acquired from the Met Office land surface observation station network (BADC, 2009).

4.8.2 Flow duration analysis

Flow duration curves (FDCs) were generated and associated indices calculated for the Lathkill and both tributaries of the Glen (Figure 5.3; Figure 6.3; Figure 6.4). FDCs show the percentage of time that river flow equals or exceeds a given value, and are therefore useful tools for setting the hydrological conditions observed during a given period in the context of the long-term average conditions. For the Glen, data from all water years (1st October to 30th September) between 1981 and 2009 were available for comparison with the study year (2007-08) for local gauging stations on both the West and East Glen (Figure 6.3; Figure 6.4). For the Lathkill, data were available from a gauging station within the study area for the period 1997-2006; however, only data for the study period (1st April-30th September) are available for the study year, rather than the whole water year (data provided by John Gunn; Figure 5.3). For each dataset, indices from Q_1 to Q_{99} (where 99 indicates flow equalled or exceeded 99 % of the time) were calculated to summarise flow characteristics (including high, median and low flow conditions) during the study year in comparison with long-term data (Table 5.3; Table 6.2).

4.9 Data analysis

4.9.1 Invertebrate community metrics

Following invertebrate sample processing and identification, four metrics were calculated for each benthic and hyporheic sample and thus used to summarise temporal and spatial variability in the community: total invertebrate abundance (TIA), taxon richness, Simpson's diversity index, and the Berger-Parker dominance index. TIA is simply the total number of invertebrates (of all taxa) present, and was supplemented by determination of the abundance of common individual taxa. Taxon richness refers to the number of taxa present in a sample, identified to the levels stated in section 4.4.5 and therefore including some group-level taxa which probably contained multiple representatives. Unidentified taxa were assigned to the most likely species where necessary to avoid overestimation of taxon richness, whilst multiple life stages were considered as individual taxa as they may occupy different ecological niches. Simpson's diversity index (Simpson, 1949) describes the probability of a second individual drawn from a population being the same species as the first, and therefore incorporates two components of community diversity: taxonomic richness and evenness. In this study, Simpson's reciprocal index 1/D was used, with 1 being the lowest possible figure and higher values reflecting higher community diversity. Berger Parker dominance (Berger and Parker, 1970) is a measure of the proportion of the community accounted for by the most common taxon which is considered an effective measure of community dominance (May, 1975).

Taxon richness, Simpson's diversity and Berger-Parker dominance were calculated using the program Species Diversity and Richness 3.03 (Pisces Conservation Ltd., 2002). Unless otherwise stated, all indices are presented per sample, i.e. per 0.1 m^2 benthic Surber sample or per 6 L hyporheic pump sample. However, to facilitate comparison with previous studies, abundance of individual invertebrate taxa is in some cases also expressed per m^2.

4.9.2 Analysis of variance (ANOVA)

Parametric ANOVA provides a powerful tool for determining deviations from the null hypotheses of no significant different over time or space, but the validity of results relies on three key assumptions being met: 1. Independence of data within and among samples; 2. Homogeneity of variances for each population; and 3. Normality of data distribution (Underwood, 1997). To determine if these assumptions were met by datasets in the current investigation and to address any violations, the following steps were taken:

Assumption 1. Independence of data among samples

Spatial autocorrelation is a form of pseudoreplication (Hurlbert, 1984) that results from the tendency of most natural variables (both environmental and biological) to be more similar over short geographical distances compared with greater distances (Legendre and Legendre, 1998). Data shown to be autocorrelated pose a problem when conducting statistical tests, as the assumption of independence is violated. To test for the presence of spatial autocorrelation in the Lathkill and Glen samples, Mantel's tests were conducted for the community datasets (Mantel, 1967; Legendre and Fortin, 1989). Mantel's tests assess correlation between two distance matrices, in this case an ecological distance matrix generated for species abundance data using an ecologically robust distance measure such as the Bray-Curtis index (Bray and Curtis, 1957; Faith *et al*., 1987), and also a spatial dissimilarity matrix of the geographic distances between each pair of sites. Separate tests were conducted for each month, to prevent the effects of temporal changes obscuring spatial patterns. In almost all months, Mantel's tests demonstrated highly significant (p <0.01) spatial autocorrelation on both rivers, and therefore repeated measures (RM) ANOVA tests were used to analyse temporal changes in all environmental and biological variables. RM ANOVA takes into account correlation between repeated measures (i.e. samples taken at the same locations at intervals during a temporal sequence) and therefore has no assumption of independence.

Assumption 2. Homogeneity of variance

Levene's tests (Levene, 1960) were incorporated into non-RM ANOVA tests to determine homoscedasticity. Where the Levene's test statistic was significant (p <0.05), this indicated that the assumption of homogeneity of variance for each population had not been met. In practice, few non-RM ANOVA tests were performed and no action was required to address violation of this assumption. In RM ANOVA tests, there is no assumption of homoscedasticity; instead, the assumption of sphericity must be met, i.e. that correlations are equal and variances are equal for all dependent variables. Sphericity can be assessed using Mauchly's tests (Mauchly, 1940), where a significant Mauchly's W statistic (p <0.05) indicates violation of this assumption. Where the assumption of sphericity was violated, estimates of sphericity (epsilon statistics) were consulted. Where epsilons exceeded 0.75, the results of Greenhouse-Geisser tests (Geisser and Greenhouse, 1958) were examined to determine significant differences between dependent variables, and where epsilons were <0.75, results of the Huynh-Feldt test were consulted (Huynh and Feldt, 1976; Quinn and Keough, 2002).

Assumption 3. Normality of data distribution

Environmental and biological variables were transformed as required to improve normality of the distribution and thus meet the assumptions of subsequent parametric analyses. Both square-root and log transformations may be suitable for ecological datasets (Zar, 1984; Underwood, 1997) and the results of both were therefore assessed for each dataset. Skewness, a measure of symmetry within a dataset, was used to select the most appropriate transformation, which in the majority of cases was the square-root transformation.

Post-hoc multiple comparison tests

Where ANOVA indicated an overall significant difference between groups, *post-hoc* multiple comparison tests were conducted to identify where these differences occurred. For one-way ANOVA, Tukey's Honestly Significant Difference (HSD) tests (Tukey, 1953) were used, and for RM ANOVA, paired *t*-tests with Bonferroni adjustments (Bonferroni, 1936) were consulted. Whilst these tests were conducted to allow description of significant patterns, the *p* values presented refer to overall significance rather than results of multiple comparisons, unless otherwise stated.

Analytical procedures

RM ANOVA tests were conducted to examine the significance of spatial and temporal changes in environmental variables, community metrics, invertebrate abundance and multivariate analysis axis scores. Normal (non-RM) ANOVA tests were conducted only for axis scores from detrended canonical correspondence analysis in which site had been included as a covariable, as these scores had already been corrected to account for site-specific variation (see below).

Two-way RM ANOVAs were conducted separately for benthic/surface and hyporheic environments with three spatial parameters as between-subject factors: site, flow permanence group (intermittent or perennial) and on the Glen, tributary (West Glen or East Glen). These two-way tests were conducted firstly, to determine significant spatial differences, and secondly to determine the significance of the interaction between the spatial parameter and the variable of interest. Whilst tests with all between-subject factors were conducted, only significant and meaningful spatial differences are presented. In addition, two-way RM ANOVAs were conducted for combined benthic-hyporheic datasets with surface water/hyporheic depth (10 cm, 20 cm, 30 cm) as a between-subject factor to identify significant differences between samples from each environment. Where no significant difference was found between hyporheic depths and no significant interaction occurred (as was typically the case), all depths were pooled prior to subsequent analyses. Regardless of significance, however, benthic/surface water and hyporheic data were analysed separately, due to the different sampling techniques used. Following completion of spatial analysis using two-way RM ANOVAs, one-way tests were used to investigate significant temporal (between-month) variation.

Data were not collected from Lathkill site 5 in May, which was problematic as datasets with missing values cannot be included in RM ANOVA tests. To resolve this, analyses of both temporal and spatial variability were conducted first for sites 1-4, to allow inclusion of May data, then repeated using June to September data to allow inclusion of site 5. In most cases these tests produced similar results and therefore, unless otherwise stated, patterns of spatial variability are presented with the exclusion of May data, whilst patterns of temporal change exclude site 5; 'all sites' refers to sites 1-4 in this context. Exceptions to this generalisation are highlighted.

All statistical tests were conducted in SPSS version 16.0 (SPSS Inc., 2007). This program was also used to generate clustered error bar plots to illustrate patterns of temporal change. Clustered error bar plots have the advantages of recognising both site-specific differences and summarising general patterns in the dataset.

4.9.3 Unconstrained ordination

Unconstrained ordination techniques (also termed indirect gradient analysis) are a type of multivariate analysis that can be used as a preliminary data exploration tool to identify the principal spatial and temporal variation in a dataset. Potentially important gradients requiring further investigation can thus be detected. Different techniques are required for investigation of invertebrate community composition and in environmental conditions, due to assumptions made regarding the underlying data structure.

Choice of approach

Unconstrained ordination techniques can be metric or nonmetric, these two approaches being fundamentally different and capable of producing contrasting solutions (Ruokolainen and Salo, 2006). No consensus has been reached regarding the superiority of either approach in a particular situation (Kenkel and Orlóci, 1986; Ruokolainen and Salo, 2006), and therefore, preliminary ordinations of invertebrate community data were conducted using one metric and one non-metric technique: detrended correspondence analysis (DCA) and non-metric multidimensional scaling (nMDS, conducted in PC-ORD; McCune and Mefford, 2006). Results from these analyses were subsequently compared, which indicated that the techniques produced similar ordinations. Therefore, only DCA was used in subsequent analyses; this procedure was favoured to facilitate comparison with other multivariate approaches used, namely principal components analysis (PCA; see below) and canonical correspondence analysis (CCA; section 4.9.4).

Detrended correspondence analysis of invertebrate community data

Correspondence analysis (CA) is a metric (eigenanalysis) ordination technique related to a unimodal response model, in which any species occurs within a limited range of each (unknown) environmental variable. DCA is based on CA (i.e. reciprocal averaging algorithm; Hill, 1973), but was developed to correct the latter's two main flaws: the *arch effect* (a mathematical artefact resulting from a lack of independence between successive axes) and the *edge effect* (pairs of samples with equivalent similarity appear farther apart at the centre of the first axis) (Hill and Gauch, 1980; Ruokolainen and Salo, 2006). CA and its derivatives order samples along multiple axes according to the frequency of co-occurrence of taxa, so that successive axes explain a declining amount of variance in the species data; the importance of each axis is measured by its eigenvalue.

In the current investigation, benthic and hyporheic communities were analysed separately as they had been sampled using different techniques. Hyporheic communities at 10, 20 and 30 cm were also initially analysed separately, with hyporheic depths subsequently being pooled due to observation of similar patterns. For each analysis, data were square-root transformed when setting ordination parameters, to reduce skewness and reduce the influence of dominant taxa (Legendre and Legendre, 1998). Preliminary analyses indicated that this was the most effective transformation, due to the high number of zero values in the hyporheic data sets. The chi-square distance measure used in correspondence analysis and its derivatives can cause rare taxa to have a disproportionately great influence on the ordination (Legendre and Gallagher, 2001), and therefore rare taxa were downweighted in all DCA ordinations (ter Braak and Šmilauer, 2002). Ordinations were performed using CANOCO for Windows version 4.54 (ter Braak and Šmilauer, 2006).

Principal components analysis of environmental data

Like DCA, PCA is an eigenanalysis ordination method, but relates to a linear rather than a unimodal response model and as such is more appropriate for analysis of environmental data (Kent and Coker, 1992). PCA creates linear combinations of the environmental variables of interest (termed principal components (PCs) or axes), so that the first PC explains the greatest variation in the dataset, and so on for successive axes (Zuur *et al*., 2007). Eigenvalues are calculated which represent the relative contribution of each axis to the explanation of the variation in the dataset.

PCA was conducted separately for surface and hyporheic water; in the former, hydrological parameters (depth, width, velocity) could be included in addition to water physicochemistry variables (pH, conductivity, temperature, DO). PCA cannot cope with missing values (Zuur *et al*., 2007), so variables (or occasionally samples) with incomplete datasets were excluded unless missing values could be extrapolated from the available data. Negative data values cause PCA to fail and therefore sub-zero dissolved oxygen values were increased to 0.1 mg L^{-1} and 1 %; such values were few and theses replacements remained well below other recorded values. In addition, notable outliers that obscured observation of general patterns were normalised. Data were standardised by dividing the variable scores by the standard deviation, to prevent species with a large variance from dominating the resulting output. Data were log-transformed to reduce skew. The species (i.e. environmental variables) data table was centred and standardized by species as the environmental variables were measured in different units (Lepš and Šmilauer, 2003). Analyses were performed using CANOCO for Windows version 4.54 (ter Braak and Šmilauer, 2006).

4.9.4 Constrained ordination

Constrained ordination, also termed direct gradient analysis, is a multivariate approach which differs from unconstrained approaches by incorporating correlation and regression between species and environmental data into the analytical procedure (ter Braak, 1986; Kent and Coker, 1992). Constrained approaches can therefore be used to explore relationships between environmental variability and biotic communities.

Canonical correspondence analysis (CCA)

The constrained ordination approach of CCA was used to examine relationships between both benthic and hyporheic invertebrate communities and hydrological variability in both rivers. CCA uses multiple regression to select the combination of environmental variables explaining the greatest amount of variation in the species data. It can therefore be used to analyse relationships between community composition and environmental variables, based on a unimodal response model. Canonical eigenvalues measure the importance of each axis and thus indicate the strength of the gradients underlying the structure in the dataset. In addition, CCA calculates both the percentage of the total variation in that dataset that is explained by each axis, and also the amount of variation explained by the axes as a fraction of the total explainable variation.

CCA was conducted separately for benthic and hyporheic habitats, and as described for PCA, hydrological variables (depth, width, velocity) could be included in addition to water physicochemistry factors (pH, conductivity, temperature, DO) in the analysis of benthic communities/surface water. Preliminary CCA was conducted to examine variance inflation factors and thus identify autocorrelated variables, which were removed as necessary. A second preliminary CCA including a forward selection procedure using 999 iterations of the Monte Carlo random permutation test was then used to examine the contribution of each retained variable to the explanatory power of the model. Two CCA ordinations were then performed, one without site as a covariable, which allowed observation of spatial patterns, and a second with site as a covariable, to facilitate examination of general patterns of temporal change. Prior to each analysis, environmental data were treated as described for PCA and community data were dealt with as described for DCA. Detrending was conducted where an arch effect (as described in DCA) was observed. Analyses were performed using CANOCO for Windows version 4.54 (ter Braak and Šmilauer, 2006).

4.9.5 Pearson's correlation coefficients

Pearson's correlation coefficients measure the strength of the correlation (linear dependence) between two variables of interest. In the current investigation, correlation coefficients were used to examine the strength and significance of relationships between: i) environmental variables and abundance of dominant taxa; ii) relationships between sediment variables and community metrics; and iii) relationships between multivariate axis scores and abundance of dominant taxa. All tests were conducted in SPSS version 16.0 (SPSS Inc., 2007).

4.9.6 Characterisation of hyporheic zone usage by benthic invertebrates

Defining a particular taxon as specifically 'benthic' or 'hyporheic' is problematic given the intimate connectivity between these two adjacent habitats. Therefore, for the purposes of this investigation, a benthic taxon was defined simply as one that occurred primarily in Surber samples collected from the surficial sediments and had no particular requirement to enter the hyporheic zone during its lifecycle. All Insecta were therefore excluded from this definition since many taxa enter the hyporheic zone during specific life stages and changes in their benthic and hyporheic abundance occur seasonally, irrespective of hydrological changes (Williams and Feltmate, 1992; Elliott, 2006). Whilst somewhat arbitrary, given that different sampling techniques were used to collect invertebrates from the two environments, this definition was suitable in light of the aims of the investigation.

To assess temporal change in the use of the hyporheic zone by benthic invertebrates, the proportion of the total (benthic + hyporheic) community within the hyporheic zone (i.e. the *hyporheic proportion* of the community) was calculated for each month. Using proportional data allowed comparison of benthic and hyporheic populations sampled using different techniques. The hyporheic proportion was calculated for the total invertebrate community (i.e. all taxa) and also for selected benthic taxa (as defined above). This analysis was necessary for subsequent comparison with changes in the benthic and hyporheic abundance, in order to infer invertebrate behaviour, with concurrent increases in hyporheic proportion and hyporheic abundance providing the most compelling evidence of active refugium use (i.e. shelter-seeking behaviour - see section 7.11 and Table 7.4).

4.10 Summary

In this chapter, the field sampling strategy, laboratory analyses and methods of data analysis that will be used to examine the response of invertebrate communities to hydrological variability have been outlined. Implementation of the described data collection and analytical procedures will address thesis aims 1 and 2 (section 1.2; Table 4.1), as detailed in Chapters 5 and 6. In turn, synthesis of the new results acquired using the outlined strategies with existing literature will facilitate the development of conceptual models, thus addressing the third thesis aim (section 1.2; Table 4.1; Chapter 7).

5. Invertebrate community response to flow variability: the River Lathkill

5.1 Introduction

In this chapter, the responses of benthic and hyporheic invertebrate communities to surface flow variability in the River Lathkill are described. Discharge in the Lathkill is groundwater dominated and flow can remain stable for prolonged periods; however transmission losses to underlying mine workings can exacerbate low flow conditions and cause streambed drying, whilst rapid-onset high-flow events can occur once the capacity of the aquifer is exceeded. Key hydrological events during the May-September 2008 study period comprised an uninterrupted four-month flow recession resulting in partial streambed drying, and a high-magnitude spate. A total of 384 invertebrate samples were collected for this research element over a period of five months, of which ¼ were from the benthic sediments and an additional ¼ from each of three depths in the hyporheic zone. A second sampling campaign was conducted at a single site in 2009 for comparative purposes, with flow remaining moderate throughout this second five-month period. In this chapter, temporal change in the composition and vertical distribution of invertebrate fauna is related to changes in primary surface hydrology and secondary environmental and biotic parameters. In particular, use of the hyporheic zone by benthic invertebrates is examined and related firstly to temporal changes in hydrological, environmental and biotic conditions, and secondly to spatial variability in relatively stable habitat parameters (hydrological exchange and sediment composition).

5.2 Aims and objectives

This chapter examines how the composition of invertebrate communities in the benthic and hyporheic sediments of the River Lathkill is affected by variation in surface flow. The specific aims and objectives of this chapter are:

Aim 1: Identify hydrological conditions and related changes in both environmental and biotic variables with the potential to cause stress in benthic invertebrates.

Objectives

1. Examine variation in surface hydrology, using discharge data and measurements taken at sampling locations.
2. Set in context the hydrological conditions experienced using long-term data.
3. Determine changes in habitat availability resulting from variation in surface flow.
4. Investigate changes in water quality parameters related to surface flow variation.
5. Use multivariate analyses to determine the principal environmental gradients.
6. Identify hydrologically-mediated changes in taxon abundance with the potential to alter the strength of biotic interactions (e.g. predation and competition) in the benthic sediments.

Aim 2: Examine benthic invertebrate community responses to identified environmental and biotic potential stressors, including changes in the use of the hyporheic zone.

Objectives

1. Investigate temporal change in invertebrate community composition in benthic and hyporheic sediments using community metrics and multivariate analysis.
2. Identify temporal changes in the abundance of common benthic taxa in the surface sediments and the hyporheic zone.
3. Analyse temporal changes in the proportion of the total (benthic + hyporheic) community inhabiting the hyporheic zone.
4. Examine relationships between environmental conditions and community metrics to determine drivers of community change.
5. Investigate spatial variability in the suitability and use of the hyporheic refugium, with reference to historic flow characteristics (including flow permanence), stable habitat parameters and temporally variable environmental factors.

5.3 Meteorological conditions and hydrological response

To address aim 1 (objective 2), the meteorological conditions experienced during the study are described and set in context by comparison with long term average (LTA) data. Streamflow response to rainfall is also considered (aim 1, objective 1).

5.3.1 Air temperature

Following an increase between early April and early May, air temperature remained relatively stable during the study (Figure 5.1), with monthly means at Wardlow Hay Cop (section 4.8.1) varying from 10.9 °C in May up to 13.8 °C in August. The lowest minimum hourly temperatures occurred in mid-May, then minima increased until August. Maximum air temperatures exceeded 20 °C each month from May to August, but such temperatures were only common in late July (Figure 5.1).

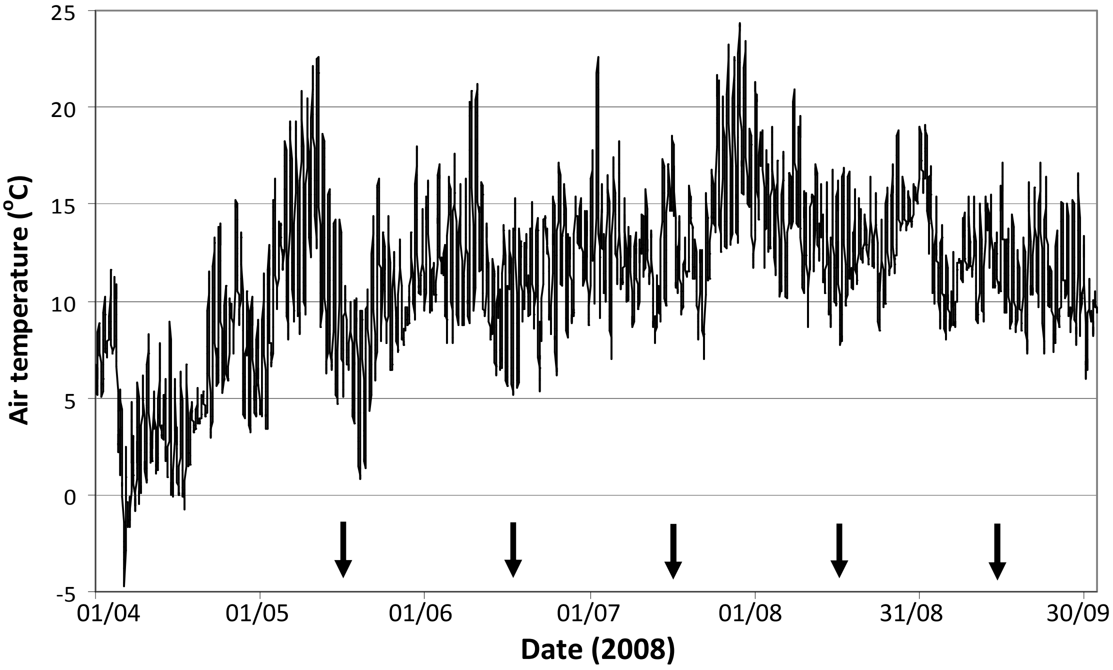


Figure 5.1: Hourly mean air temperature at Wardlow Hay Cop (SK178739; see Table 5.2), April to September 2008. Arrows indicate sampling dates.

Wardlow Hay Cop data were compared with 2003-2007 mean values for Keele, a Met Office station 44 km south-west of the Lathkill at a similar elevation (Met Office, 2009a). This comparison indicated that May temperatures were slightly above average whilst April and June to September temperatures were below average (Met Office, 2009d); this pattern was also reported by Hilton (2009) at Buxton, 14 km north-west of the Lathkill and at a slightly higher elevation (~100 m; Table 5.1).

Table 5.1: Mean monthly air temperature at Buxton, April-September 2008, in comparison with the 2003-2007 mean

	Mean monthly air temperature (^{o}C)*	
	2003-2007 mean	2008
April	7.81	5.7
May	10.1	11.9
June	13.4	12.6
July	15.6	14.7
August	14.8	14.6
Sept	13.8	11.7

*Data from Hilton (2009), from SK056707, 14 km north-west of the River Lathkill.

5.3.2 Rainfall and streamflow response

Prior to the study commencing, streamflow in the Lathkill decreased sharply in April during a month of below-average rainfall (Figure 5.2; Table 5.2). Rainfall then remained close to the long-term average (LTA, 1991-2000) in May and June, and consequently streamflow continued to decline gradually. In July, August and September, rainfall greatly exceeded the LTA, approaching double the 1991-2000 mean in August (Table 5.2). Despite this, flow recession continued uninterrupted until mid-August as precipitation inputs recharged the groundwater aquifer, with the

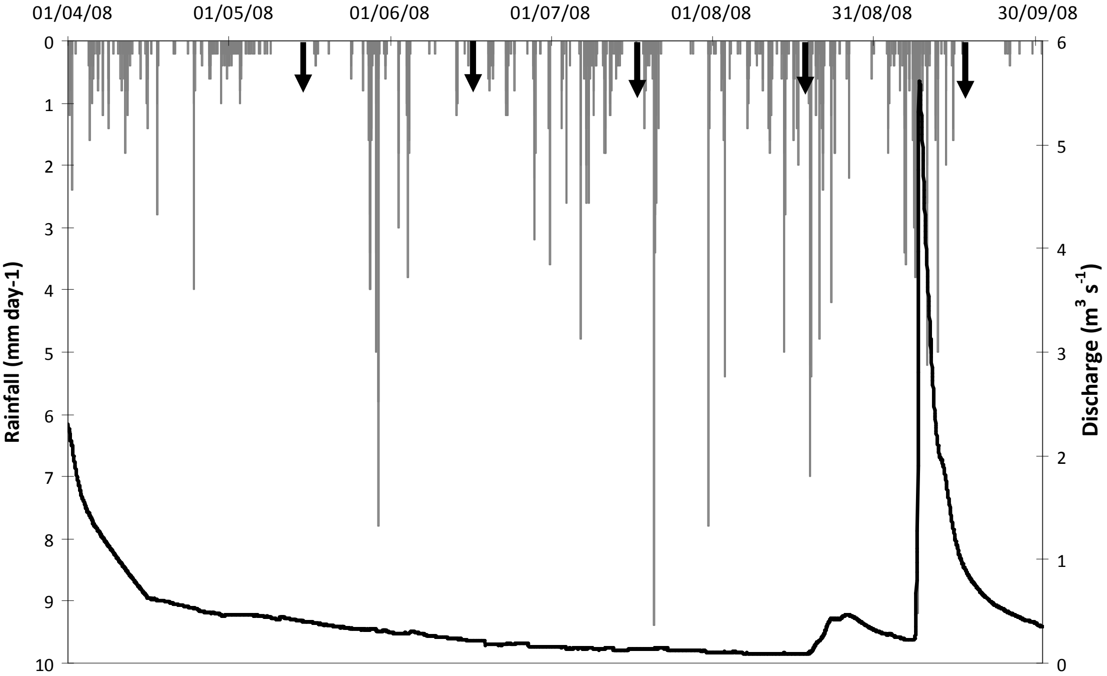


Figure 5.2: Hourly hydrograph for the River Lathkill at Psalm Pool (SK205661, 1 km downstream of the study reach) and rainfall at Wardlow Hay Cop (SK178739; see Table 5.2), April to September 2008. Arrows indicate sampling dates.

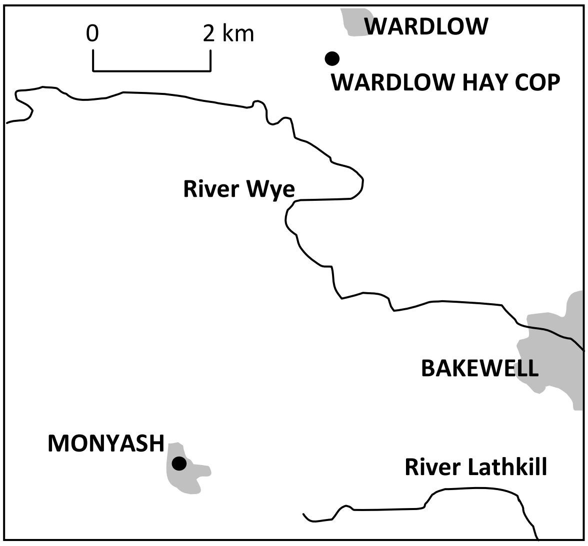
lowest discharge being recorded on 15th August. A fivefold increase in streamflow then occurred in late August, which preceded a high-magnitude spate event on 6th September during which discharge rose from <0.23 to >5.6 $m^3 s^{-1}$ within 30 hours. Final sampling was undertaken in mid-September as spate flows receded (Figure 5.2).

Table 5.2: Mean monthly rainfall in the River Wye catchment, April-September 2008, compared with the 1991-2000 mean. Locations of gauging stations are shown in relation to the River Lathkill

	Rainfall (mm day^{-1})	
	Wardlow Hay Cop 2008*	**Monyash 1991-2000****
April	65.2	87.7
May	65.8	60.7
June	64.6	81.2
July	104.4	59.6
August	118.2	57.9
Sept	129.4	77.0

* Provided by the Limestone Research Group, University of Birmingham.

** Met Office MIDAS land surface observation station data (BADC, 2009)



5.3.3 Flow duration analysis

Flow duration curves (FDC) were generated and related indices calculated to allow comparison of conditions during and preceding the study period with average conditions (Figure 5.3; Table 5.3). However, the only available data for this analysis (provided by John Gunn, University of Birmingham) are an incomplete, pre-analysed dataset for a site within the study reach (as opposed to the downstream discharge data presented in Figure 5.2). The study period (1st April-30th September 2008) is compared with the previous ten water years (October to September 1997/98 to 2006/7).

Comparison of the upper regions of the 1997-2006 and study period FDCs (Figure 5.3) indicated that the September spate was not particularly high magnitude, although reference to hydrographs for earlier periods (data not presented) showed that the rate of flow increase was unusually rapid, and high magnitude spates are uncommon during the summer months (P. Bowler, pers. comm.). The steep slope in the upper region of the FDC, whilst suggesting that the decline to low flow conditions was more rapid than is typical, probably reflected the exclusion of winter data from 2008. Considering low flows, discharge levelled off rather than continuing to decline to zero, despite notable occurrence of such conditions in preceding years (Figure 5.3; Table 5.3).

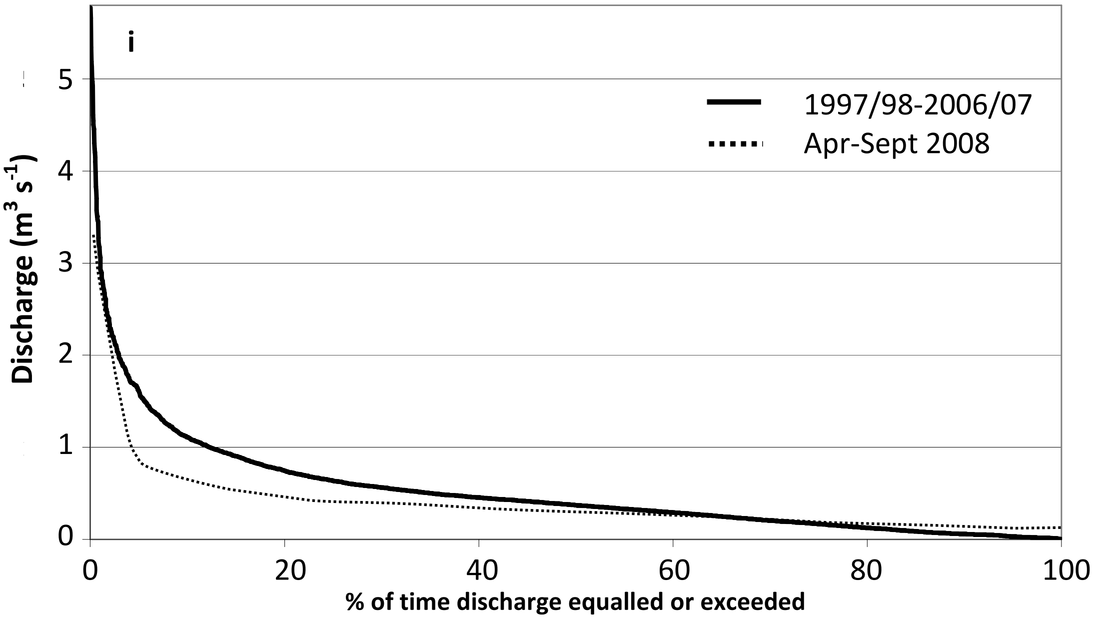


Figure 5.3: Flow duration curves for the River Lathkill study area. Mean daily discharge equalled or exceeded 0-100 % of the time. Data presented as supplied by John Gunn, University of Birmingham.

Table 5.3: Indices derived from flow duration analysis, indicating discharge during the study in comparison with the long term average

	Mean daily discharge ($m^3\ s^{-1}$)	
Index	**1997-2006***	**April-Sept 2008**
Q_1	~2.2	2.9
Q_5	~1.6	0.87
Q_{10}	~1.1	0.56
Q_{30}	~0.55	0.31
Q_{50}	~0.35	0.22
Q_{90}	~0.06	0.08
Q_{95}	~0.03	0.07
Q_{99}	0	0.07

***Values estimated from flow duration curve**

5.4 Spatiotemporal variability in environmental conditions

To address aim 1, hydrological variables measured instream are examined (objective 1) and the effects of these changes on submerged habitat availability are determined (objective 3). Associated changes in water quality parameters are also considered (objective 4), then principal components analysis is then used to identify the main environmental gradients in the data (objective 5).

5.4.1 Variation in surface hydrology

To supplement the continuous discharge data, measurements of water depth and mean flow velocity (at 0.6x depth) were made at each sampling point each month to characterise the effects of discharge variability on instream habitats. Width was determined *post hoc* by applying depth data to cross-sectional channel profiles.

Surface water depth, flow velocity and wetted width

Depth was comparable at sites 1-4 and higher at site 5 ($F_{4, 15} = 14.578$, $p \leq 0.001$; Table 5.5). Depth decreased between May and June, remained stable in July, fell to the lowest recorded values in August (zero at two sampling points), then peaked in September ($F_{2.693, 51.164} = 52.338$, $p < 0.001$; Table 5.4; Figure 5.4(i)). The interaction between depth and site was significant ($F_{12, 48} = 7.290$, $p < 0.001$), with the overall pattern being particularly apparent at site 1. At site 2, depth declined between May and June, remained low between June and August then peaked in September; at site 3, depth declined gradually between May and August then peaked in September; and at sites 4 and 5, depth was similar between May and July, fell sharply in August then increased to a September peak. Natural hydrological variability was influenced by the operation of a downstream sluice gate at site 4 and, in particular, site 5. Temporal variability was significant at all sites ($F \geq 13.887$, $p \leq 0.005$).

Mean velocity was comparable at sites 1-4 but much lower at site 5 ($F_{4, 15} = 5.490$, $p = 0.006$; Table 5.5). Velocity declined gradually between May and July-August then increased dramatically in September ($F_{2.359, 35.390} = 20.891$, $p < 0.001$; Table 5.4; Figure 5.4(ii)). The highest individual flow velocities (1.16 m s^{-1}) were recorded at site 3 in September, whilst measurable flow ceased at two intermittent sampling points in August. Interactions with velocity were not significant for spatial parameters. However, reductions in velocity were particularly pronounced at site 4, with some sampling areas being almost ponded in August (Figure 5.4(ii)).

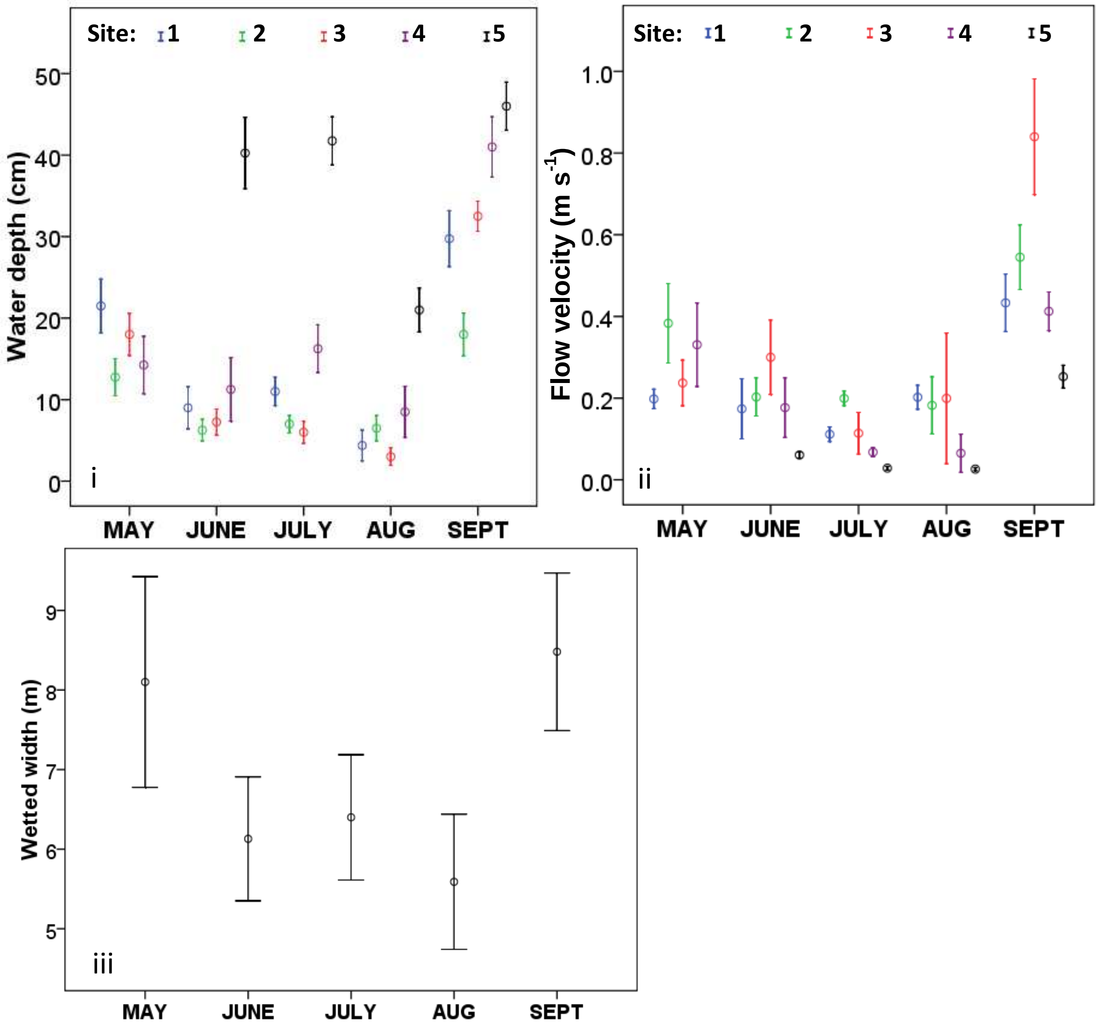


Figure 5.4: Mean ± 1SE temporal change in hydrological variables: i) water depth; ii) mean (0.6x depth) flow velocity; iii) wetted width (pooled data from all sites, see section 4.5.2).

Table 5.4: Temporal change in surface water hydrology on the River Lathkill, May - September 2008

Hydrological variables	May	June	July	August	Sept	Temporal change§
Surface water depth	16.6 ± 1.6	8.4 ± 1.2	10.1 ± 1.3	5.6 ± 1.1	30.3 ± 2.5	**
Mean flow velocity (m s^{-1})	0.29 ± 0.04	0.21 ± 0.04	0.12 ± 0.02	0.16 ± 0.04	0.6 ± 0.06	**
Wetted width	8.1 ± 1.3	6.1 ± 0.8	6.4 ± 0.8	5.6 ± 0.8	8.5 ± 1.0	**

Mean ± 1 SE of all samples. Each month, n = 16 for depth and velocity and n = 10 for width. Site 5 depth and velocity measurements are excluded to avoid biasing the dataset. §One-way RM ANOVA tests, ** indicates p <0.01.

Width was greater at perennial sites 1 and 2 than at intermittent sites 3-5 ($F_{1,8}$ = 7.809, p = 0.023; Table 5.5), but whilst all sites contributed to this pattern, width has been anthropogenically influenced and differences may not be related to flow permanence. Width declined between May and June, increased slightly in July, fell to the lowest levels recorded in August then peaked in September ($F_{2.192, 19.727} = 9.242$, $p = 0.001$; Table 5.4; Figure 5.4(iii)). The interaction with width was not significant for flow permanence group or site.

Table 5.5: Spatial differences in surface water hydrology at River Lathkill sites 1-5

Hydrological variables	Site 1	Site 2	Site 3	Site 4	Site 5	Spatial change§
Surface water depth (cm)	15.1 ± 2.4	10.1 ± 1.3	13.4 ± 2.6	18.3 ± 3.0	36.3 ± 2.4	**
Mean flow velocity (m s^{-1})	0.22 ± 0.03	0.30 ± 0.04	0.34 ± 0.07	0.21 ± 0.04	0.09 ± 0.03	**
Wetted width (m)	8.9 ± 1.3	9.9 ± 0.5	3.4 ± 0.2	7.4 ± 0.7	5.5 ± 0.2	*

Values presented as mean ± 1 SE of all samples; *n* = 20 at sites 1-4 and *n* = 16 at site 5 for depth and velocity; *n* = 5 at all sites for width. §Two-way RM ANOVA tests; ** indicates $p < 0.01$, * indicates $p < 0.05$.

5.4.2 Submerged habitat availability

Width and depth measurements were applied to cross-sectional channel profiles of each site to determine effects of temporal change on the extent of submerged benthic habitat (SBH). First, the maximum extent of SBH recorded during the study was calculated for each site (Figure 5.5; Table 5.6). The percentage of this maximum SBH that was submerged and thus available for invertebrate inhabitation (% max. SBH) was then determined for each other month. At sites 1, 2, 3 and 5, a single cross-section was representative of all sampling points, whilst two cross sections were used for site 4 due to variation in bed morphology (Appendix 7).

Table 5.6: Temporal change in the extent of submerged benthic sediments as a percentage of the maximum recorded

	Submerged % of benthic sediments at site:					
Site	**1**	**2**	**3**	**4 (1)***	**4 (4)***	**5**
May	51.3	69.4	47.0	16.7	56.9	100
June	20.2	29.2	16.3	8.1	55.4	100
July	29.7	29.2	13.8	18.6	58.4	94.4
August	17.2	15.9	7.9	5.1	47.8	19.5
September	100	100	100	100	100	100

***Bracketed numbers refer to the closest sampling point**

Without exception, the max. SBH was recorded in September, but temporal changes in the preceding months varied within and between sites depending on bed morphology (Table 5.6; Appendix 7). At sites 1 and 2, % max. SBH dropped sharply between May and June following exposure of mid channel sediments (Figure 5.5(i)) and further declines in August were relatively minor (Table 5.6). The % max. SBH also fell sharply between May and June at site 3, then continued to decline gradually, with only 7.9 % of the recorded maximum available by August (Table 5.6). In the downstream area of site 4 (4, 1), the % max. SBH was very low from May onwards and fell to only 5.1 % in August. In contrast, the channel morphology in the upstream part of this site (4, 4; compare Figure 5.5(ii) and (iii)) allowed the % max. SBH to remain much higher in all months (Table 5.6). At site 5, the % max. SBH approached 100 % until a sharp drop in August. Therefore, at all sites, habitat availability was lowest in August and increased sharply in September (Table 5.6).

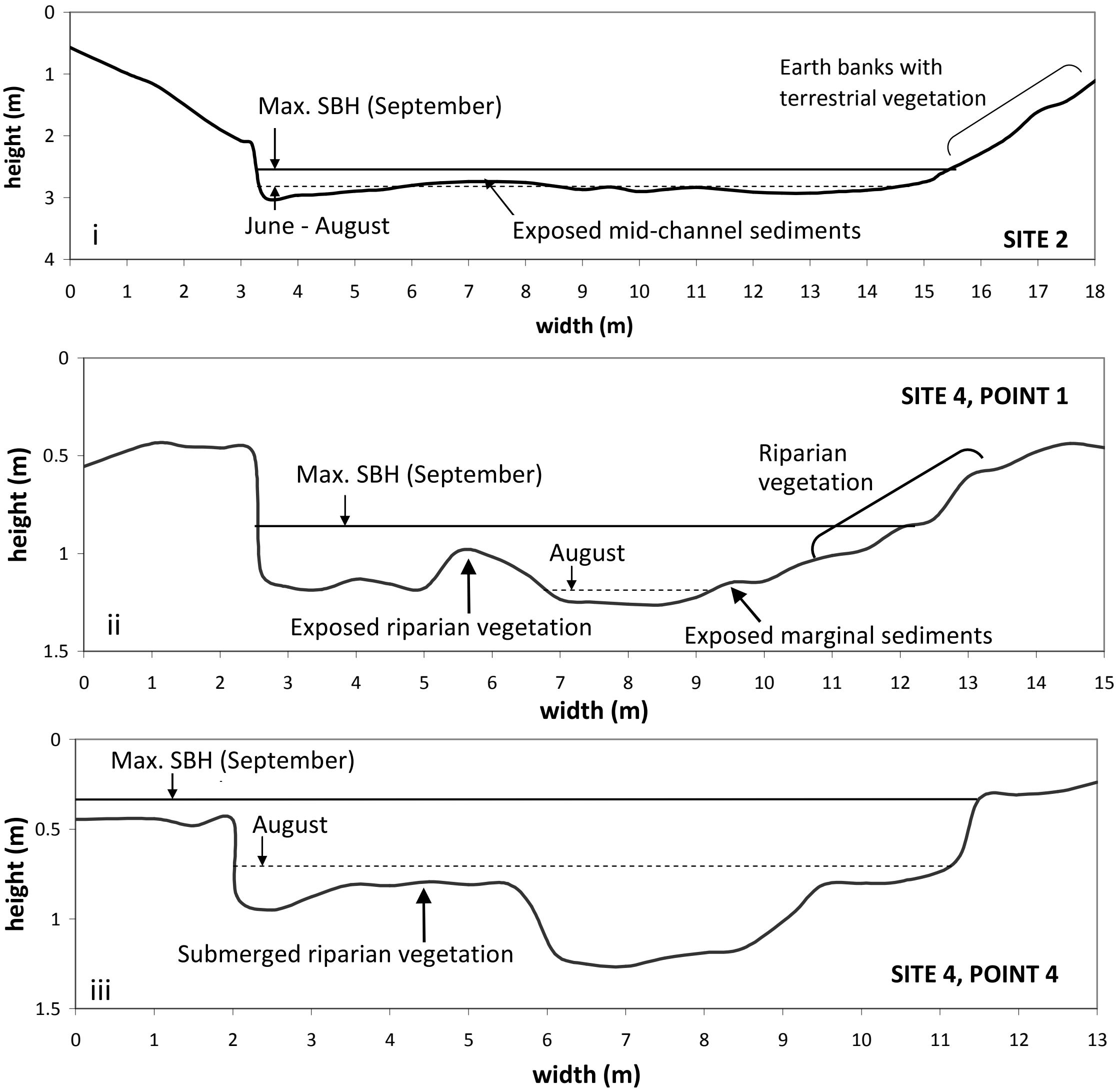


Figure 5.5: Cross-sectional profiles illustrating changes in the extent of submerged benthic sediments in relation to water depth: i) site 2; ii) site 4, sampling areas 1/2; iii) site 4, sampling areas 3/4. Key: Max. SBH = maximum extent of submerged benthic habitat.

5.4.3 Water quality

Water quality parameters that may have changed in response to variation in surface flow were investigated; mean monthly values are presented in Table 5.7. Preliminary analyses found very few differences between hyporheic depths and all depths were pooled for analysis. Spatial variability was considered between historic flow permanence groups (intermittent, perennial) and between sites; for brevity, non-significant patterns are not always described and non-significant results not always stated, $p > 0.05$ in all cases.

Dissolved oxygen

No DO data is available for September and insufficient measurements were collected in May to include these months in analyses. Both concentration and % saturation were determined and values are presented in Table 5.7. As both measures followed similar spatiotemporal patterns, only DO concentrations will be described, in which temporal variation was more pronounced.

Surface water DO concentrations were low at site 1 compared with other sites ($F_{1,2} = 894.090$, $p = 0.001$; Table 5.8). Considering all sites, mean concentrations declined between June and August ($F_{2,36} = 3.043$, $p = 0.060$; Figure 5.6(i)), with the lowest individual value, 5.9 mg L^{-1}, being recorded at site 1 in the latter month. The interaction with DO was significant for site ($F_{4,14} = 33.473$, $p < 0.001$). At sites 1, 3 and 5, DO concentrations were highest in June, declined in July then remained low in August; at site 4, concentrations increased gradually between June and August; and at site 2, concentrations were particularly high in July. Temporal change was significant at all sites ($F \geq 6.747$, $p \leq 0.029$). The few values available for May indicate that DO concentrations were higher in this month than between June and August.

DO concentrations were significantly lower in hyporheic water (6.3 ± 0.2 mg L^{-1}) than in surface water (9.5 ± 0.3 mg L^{-1}; $F_{1,61} = 48.900$, $p < 0.001$). Hyporheic concentrations were lowest at site 1 and highest at site 2 ($F_{3,44} = 6.209$, $p = 0.001$; Table 5.8). Overall, hyporheic DO concentrations were similar in June and July then declined in

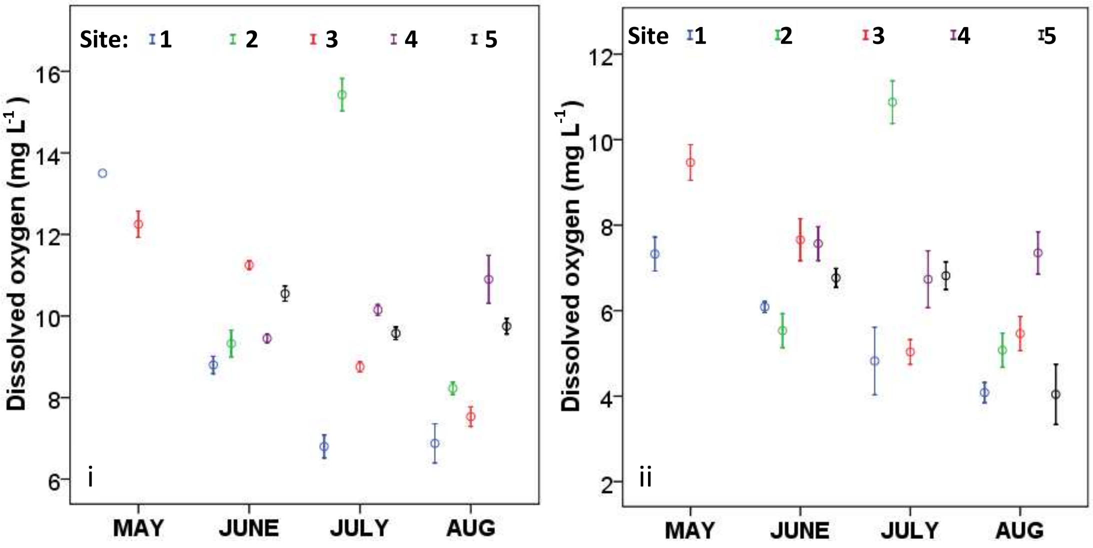


Figure 5.6: Mean ± 1 SE temporal change in dissolved oxygen concentrations (mg L^{-1}): i) surface water; ii) hyporheic water.

August ($F_{1.315, 61.782}$ = 10.587, p = 0.001; Table 5.7; Figure 5.6(ii)), with a significant interaction observed between concentration and site ($F_{6, 88}$ = 36.793, p <0.001). At site 1 (May data available), DO concentrations were highest in May then fell each month until August; at site 2, DO concentrations were highest in July; and at sites 3 and 4 concentrations were higher in June than in July and August. Temporal change was significant at sites 1-4 (F ≥5.204, p ≤0.029). Insufficient site 5 data is available for analysis; however, individual values dropped to 0.7 mg L^{-1} in August.

Water Temperature

Surface water temperature was lowest at site 1 and highest at site 5 ($F_{4, 15}$ = 147.223, p <0.001; Table 5.8). Mean temperatures were highest in May and lowest in September but remained moderate throughout the study ($F_{1.410, 26.784}$ = 11.693, p = 0.001; Table 5.7; Figure 5.7(i)), with the highest individual values reaching 13.3 ^{o}C at site 5 in July and August, and the lowest (8.7 ^{o}C) recorded at site 4 in May. Water temperature was similar in surface water (10.3 ± 0.1 ^{o}C) and at all hyporheic depths (10.5 ± 0.1 ^{o}C). Patterns of spatial variability in hyporheic temperatures reflect those reported for surface water (Table 5.8), whilst temporal variation differed, with the highest mean temperatures occurring in July ($F_{1.675, 78.718}$ = 32.064, p <0.001; Figure 5.7(ii); Table 5.7).

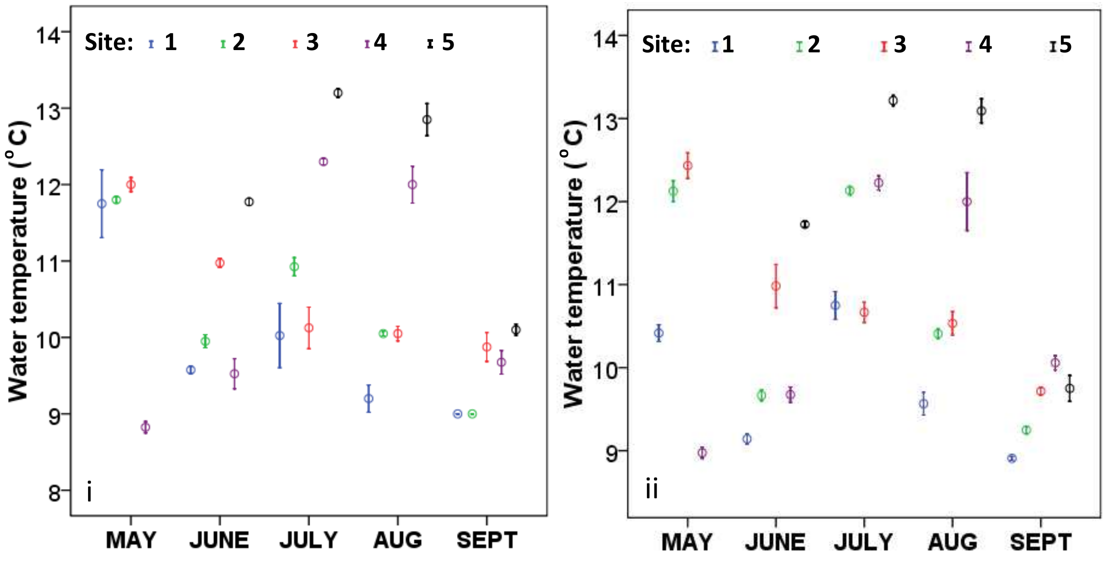


Figure 5.7: Mean ± 1 SE temporal change in temperature (°C): i) surface water; ii) hyporheic water.

Fine sediment

In surface water, fine sediment concentrations were lowest at site 1 and highest at site 5 (Table 5.8) but spatial variability was not significant. Considering all sites, concentrations were highest and most variable in August and lowest in September; temporal change was not significant ($F_{2, 8}$ = 1.875, p = 0.215; Table 5.7).

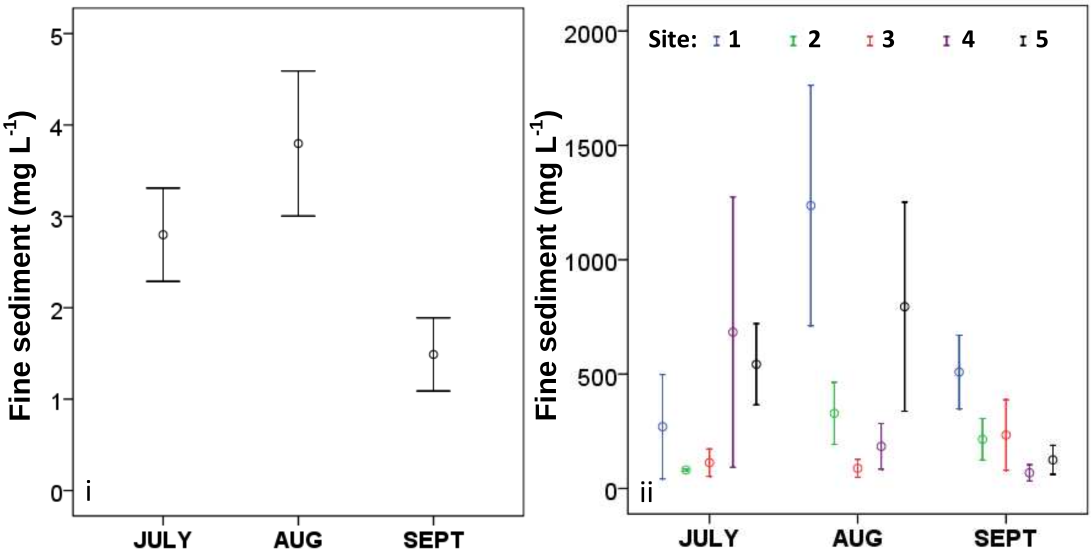


Figure 5.8: Mean ± 1 SE temporal change in fine sediment concentrations (mg L^{-1}): i) surface water; ii) hyporheic water. An outlier (hyporheic water, August, site 1) has been normalised to be in line with other samples.

Comparison of surface water and hyporheic concentrations is not valid due to different sampling techniques. In contrast to the spatial pattern observed in surface water, hyporheic fine sediment concentrations were highest at site 1, partly due to an outlier (5900 mg L^{-1}), and lowest at site 3, but spatial differences were not significant (Table 5.8). Temporally, as in surface water, hyporheic concentrations were high and variable in August, partly due to very high values (>1600 mg L^{-1}) at sites 1 and 5, and were moderately lower in September ($F_{2, 28} = 1.796$, $p = 0.185$).

Particulate organic carbon

Surface water POC concentrations were lower at sites 1, 2 and 5 and higher at sites 3 and 4 ($F_{1, 2} = 281.761$, $p = 0.004$; Table 5.8). Concentrations rose between July and August and were lowest in September ($F_{2, 8} = 5.069$, $p = 0.038$; Table 5.7). The interaction between POC concentration and flow permanence was not significant. Comparison of surface water and hyporheic concentrations is again not valid due to different sampling techniques. Hyporheic POC concentrations were very variable and values were comparable at all sites. Hyporheic concentrations were also considerably higher in August than other months but neither temporal change nor interactions with spatial parameters were significant (Table 5.7).

Nitrate and Phosphate

Nitrate concentrations were highly variable within individual months and sites. In surface water, values were higher at intermittent sites 3-5 compared with perennial sites 1 and 2 ($F_{1, 2} = 144.845$, $p = 0.007$); a similar (non-significant) pattern was observed in hyporheic water (Table 5.8). Nitrate concentrations were similar in surface water and at all hyporheic depths. Surface concentrations varied between 4.8 mg L^{-1} at site 3 in September and 0.9 mg L^{-1} at site 2 in May, but temporal change was not significant. Hyporheic concentrations were similarly low in June and August and significantly higher in July and September ($F_{3, 42} = 7.402$, $p < 0.001$; Table 5.7). Interactions with site/flow permanence group were not significant.

Surface phosphate concentrations varied between 0.01 mg L^{-1} at site 4 in May and 0.2 mg L^{-1} at site 2 in September. Concentrations were similar in surface water and hyporheic water and spatial variability in concentrations was not significant in either environment (Table 5.8). Temporally, concentrations were highest in September, but temporal change was only significant in hyporheic water ($F_{1.962, 27.467} = 6.449$, p =

0.005; Table 5.7). The interaction with site was significant for hyporheic concentrations ($F_{12, 30}$ = 2.380, p = 0.027) but temporal change was not significant at individual sites.

pH

Significant variation in pH observed between months, sites and surface/hyporheic water reflected only minor changes (Table 5.7; Table 5.8). Spatially, surface water pH was lower at site 1 than at other sites ($F_{3, 12}$ = 83.949, p < 0.001); hyporheic values were comparable at all sites (Table 5.8). Temporally, particularly low values in August may be due to equipment malfunction (Table 5.7). The lowest value, 6.4, was recorded in hyporheic water at site 1 in August, whilst hyporheic pH reached or exceeded 9 at four sites in either June and/or July.

Conductivity

In surface water, conductivity was highest at site 1 then declined with progression downstream ($F_{3, 12}$ = 13.758, p <0.001; Table 5.8). Conductivity was lowest in May then increased gradually to an August peak before returning to June levels in September ($F_{1.910, 22.917}$ = 132.962, p <0.001; Table 5.7). The highest values (659 µS cm^{-1}) occurred at site 1 in August. The interaction with conductivity was significant for site ($F_{5.729, 22.917}$ = 22.176, p <0.001), and the overall pattern was observed at sites

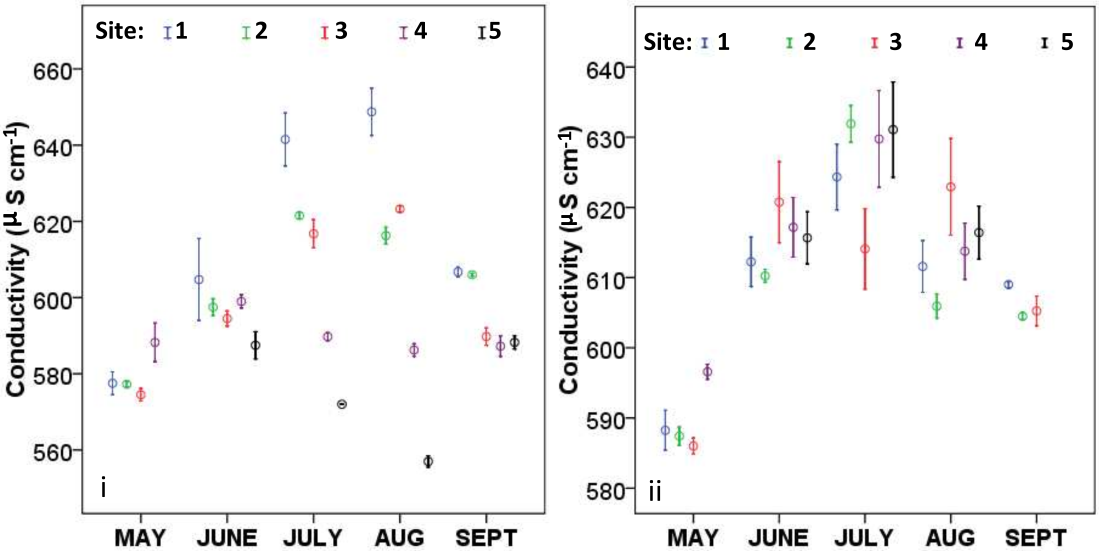


Figure 5.9: Mean ± 1 SE temporal change in conductivity (µS cm^{-1}): i) surface water; ii) hyporheic water.

1 and 3. At site 2, conductivity peaked in July; at site 4, values were similar in all months; and at site 5, conductivity declined between June and August then increased in September. Conductivity was higher in hyporheic water (612 ± 1.2 µS cm^{-1}) than surface water (602 ± 2.4 µS cm^{-1}; $F_{1, 62}$ = 15.152, p <0.001). As in surface water, hyporheic conductivity was highest at site 1 then declined with progression downstream ($F_{3, 44}$ = 57.495, p <0.001; Table 5.8). The overall pattern of temporal change reflected that seen in surface water ($F_{1.422, 66.833}$ = 75.732, p <0.001) with a gradual increase to an August peak occurring at sites 1, 2 and 3 (Table 5.7). The interaction with site was significant ($F_{8.502, 124.696}$ = 50.245, p <0.001), and at site 4, conductivity was lowest in May and August and peaked in June, and at site 5, values peaked in September.

Table 5.7: Temporal change in physicochemical measures of surface and hyporheic water in the River Lathkill, May to September 2008

Variable	Surface or hyporheic	May	June	July	August	Sept	Temporal change
Dissolved oxygen (mg L^{-1})	Surface	12.5 ± 0.4	9.9 ± 0.2	10.1 ± 0.7	8.7 ± 0.4	-	**
	Hyporheic	7.8 ± 0.4	6.7 ± 0.2	6.8 ± 0.4	5.2 ± 0.3	-	**
Dissolved oxygen (% saturation)	Surface	100 ± 0	95.5 ± 1.2	93.4 ± 1.8	91.4 ± 2.4	-	ns
	Hyporheic	85.5 ± 3.0	74.0 ± 1.7	75.8 ± 2.9	69.4 ± 2.3	-	ns
Water temperature (°C)	Surface	11.1 ± 0.4	10.0 ± 0.2	10.8 ± 0.3	10.3 ± 0.3	9.4 ± 0.1	**
	Hyporheic	11.0 ± 0.2	9.9 ± 0.1	11.4 ± 0.1	10.8 ± 0.2	9.5 ± 0.1	**
Fine sediment (mg L^{-1})	Surface	-	-	0.9 ± 0.6	4.0 ± 0.2	0.8 ± 0.4	ns
	Hyporheic	-	-	340 ± 130	790 ± 390	230 ± 60	ns
POC (mg L^{-1})	Surface	-	-	2.8 ± 0.5	3.8 ± 0.7	1.5 ± 0.4	*
	Hyporheic	-	-	73.4 ± 19	188 ± 73	47 ± 11	ns
Nitrate (mg L^{-1})	Surface	2.6 ± 0.6	2.7 ± 0.8	3.6 ± 0.1	2.2 ± 0.4	3.3 ± 0.5	ns
	Hyporheic	-	2.5 ± 0.3	3.6 ± 0.2	2.6 ± 0.2	3.6 ± 0.2	**
Phosphate (mg L^{-1})	Surface	0.05 ± 0.02	0.13 ± 0.03	0.08 ± 0.02	0.08 ± 0.02	0.14 ± 0.02	ns
	Hyporheic	-	0.15 ± 0.03	0.10 ± 0.02	0.08 ± 0.01	0.25 ± 0.05	**
pH	Surface	8.1 ± 0.06	8.2 ± 0.04	8.4 ± 0.10	7.6 ± 0.06	7.8 ± 0.03	**
	Hyporheic	8.0 ± 0.03	8.1 ± 0.05	8.3 ± 0.06	7.1 ± 0.03	7.8 ± 0.03	**
Conductivity (µS cm^{-1})	Surface	579 ± 1.9	599 ± 2.7	617 ± 5.1	619 ± 5.9	597 ± 2.5	**
	Hyporheic	590 ± 1.0	611 ± 1.5	624 ± 2.1	629 ± 3.3	607 ± 0.6	**

Values are presented as the mean ± 1 SE of all samples; $n = 16$ in surface water and $n = 48$ in hyporheic water in all months for all variables, with the following exceptions: surface water DO (mg L^{-1}), where $n = 8$ in June and $n = 7$ in September; hyporheic water DO (mg L^{-1}), where $n = 15$ in May; surface nitrate, phosphate, POC and fine sediment, $n = 4$; hyporheic nitrate, phosphate, POC and fine sediment, $n = 15$. Temporal change analysed using one-way RM ANOVA, with * and ** indicating overall significance levels of $p < 0 .05$ and $p < 0.01$ respectively, and ns indicating $p > 0.05$. Key: POC = particulate organic carbon

Table 5.8: Spatial differences in physicochemical measures of surface and hyporheic water at River Lathkill sites 1-5

Variable	Surface or hyporheic	Site 1	Site 2	Site 3	Site 4	Site 5	Spatial change
Dissolved oxygen (mg L^{-1})	Surface	8.0 ± 0.6	11.0 ± 1.0	10.1 ± 0.5	10.2 ± 0.3	10.0 ± 0.2	**
	Hyporheic	5.0 ± 0.3	7.2 ± 0.5	6.0 ± 0.3	7.0 ± 0.3	5.6 ± 0.4	**
Dissolved oxygen (% saturation)	Surface	84.2 ± 2.1	96.8 ± 1.1	93.1 ± 2.4	98.3 ± 0.8	99.3 ± 0.4	**
	Hyporheic	63.8 ± 1.9	76.5 ± 3.2	70.6 ± 1.9	79.0 ± 2.6	67.4 ± 3.4	**
Water temperature (^{o}C)	Surface	9.9 ± 0.3	10.3 ± 0.2	10.6 ± 0.2	10.5 ± 0.3	12.0 ± 0.3	**
	Hyporheic	9.8 ± 0.1	10.7 ± 0.2	10.9 ± 0.1	10.7 ± 0.3	11.9 ± 0.2	**
Fine sediment (mg L^{-1})	Surface	0.26 ± 0.26	0.47 ± 0.30	3.78 ± 1.50	4.69 ± 4.43	1.05 ± 0.55	ns
	Hyporheic	541 ± 168	208 ± 59	145 ± 53	312 ± 197	487 ± 173	ns
POC (mg L^{-1})	Surface	2.2 ± 0.72	1.9 ± 0.06	3.6 ± 1.11	3.6 ± 1.34	2.1 ± 0.86	**
	Hyporheic	192 ± 100	58 ± 17	37 ± 13	111 ± 74	117 ± 38	ns
Nitrate (mg L^{-1})	Surface	2.4 ± 0.5	2.4 ± 0.5	3.3 ± 0.5	3.2 ± 0.4	3.3 ± 0.4	**
	Hyporheic	3.1 ± 0.4	2.4 ± 0.3	3.6 ± 0.2	3.4 ± 0.2	2.8 ± 0.3	ns
Phosphate (mg L^{-1})	Surface	0.10 ± 0.01	0.10 ± 0.03	0.09 ± 0.02	0.08 ± 0.03	0.11 ± 0.05	ns
	Hyporheic	0.16 ± 0.06	0.16 ± 0.03	0.14 ± 0.03	0.14 ± 0.03	0.14 ± 0.03	ns
pH	Surface	7.7 ± 0.07	8.1 ± 0.09	8.0 ± 0.04	8.2 ± 0.09	8.1 ± 0.12	**
	Hyporheic	7.6 ± 0.06	8.0 ± 0.06	7.8 ± 0.07	8.0 ± 0.05	8.0 ± 0.07	ns
Conductivity (µS cm^{-1})	Surface	615 ± 6.5	604 ± 3.6	600 ± 4.2	590 ± 1.5	576 ± 3.4	**
	Hyporheic	622 ± 3.4	612 ± 2.0	611 ± 2.1	602 ± 1.1	590 ± 2.1	**

Values are presented as the mean ± 1 SE of all samples. In surface water, *n* = 20 at sites 1-4 and *n* = 16 at site 5, with the following exceptions: DO (mg L^{-1} and %), *n* = 13 at site 1, *n* = 12 at site 2, 4 and 5, and *n* = 15 at site 3; nitrate and phosphate, *n* = 5 at sites 1-4 and *n* = 4 at site 5; POC and fine sediment, *n* = 3 at all sites. In hyporheic water, *n* = 60 at sites 1-4 and *n* = 48 at site 5, with the following exceptions: DO (mg L^{-1} and %), *n* = 36 at sites 1-4 and *n* = 27 at site 5; nitrate and phosphate, *n* = 12 at all sites; POC and fine sediment, *n* = 9 at all sites. Spatial change analysed using two-way RM ANOVA, with ** indicating overall significance levels of $p < 0.01$ and ns indicating $p > 0.05$. Key – see Table 5.7.

5.4.4 Principal Components Analysis

Separate PCA analyses were conducted for surface and hyporheic water; in the former, hydrological parameters (depth, width, velocity) could be included in addition to water chemistry variables (pH, conductivity, temperature, DO).

Surface water

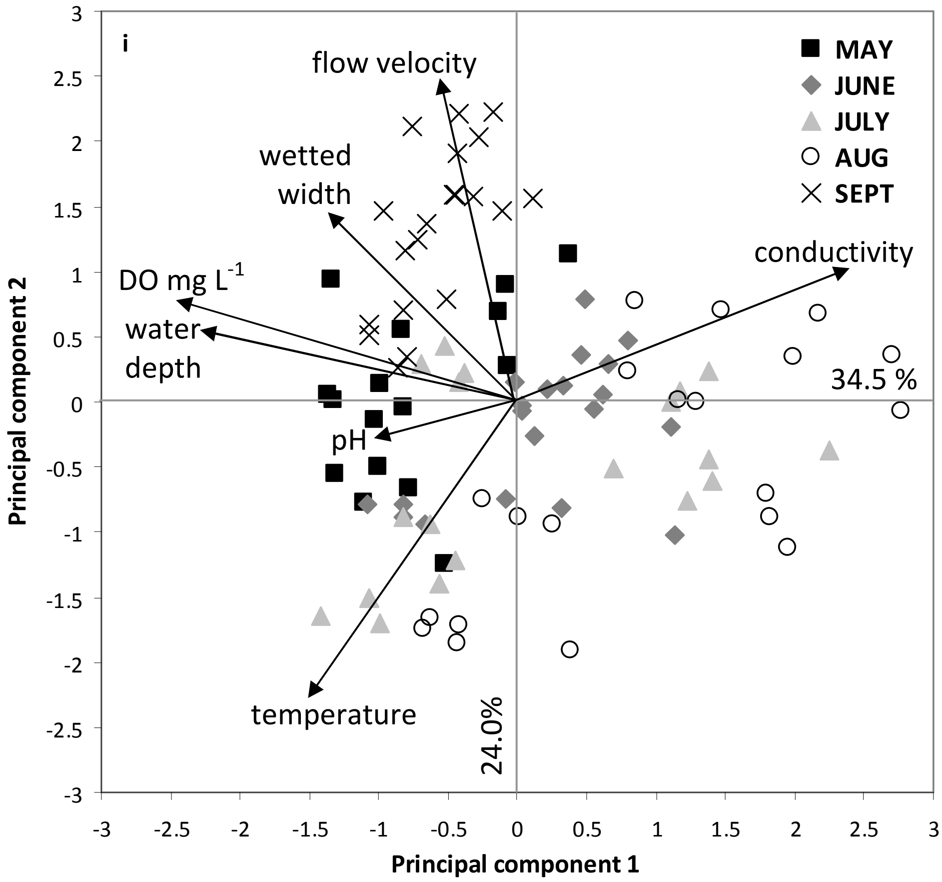
PCA ordination of surface water data is presented both by month and by site (Figure 5.10). PC1 explained 34.5 % of the variance and was most strongly correlated with conductivity (Pearson's correlation coefficient (PCC) = 0.767, p <0.001), DO concentration (PCC = -0.755, p <0.001), and depth (PCC = -0.671, p <0.001). PC2 explained an additional 24 % of the variance and had strong negative correlations with flow velocity (PCC = -0.786, p <0.001) and temperature (PCC = -0.756, p <0.001).

Despite intra-month variability and inter-month overlap (Figure 5.10(i)), temporal change was significant on PC1 ($F_{4, 91}$ = 13.029, p <0.001) and PC2 ($F_{4, 91}$ = 24.765, p <0.001). PC1 scores increased between May and August in response to decreasing DO and depth and increasing conductivity, then returned to May levels as depth and DO increased and conductivity fell in September. PC2 scores declined between May and July/ August in response to declining velocity, then rose in September as velocity peaked.

Environmental conditions were very variable at many sites (Figure 5.10(ii)) and considerable overlap was apparent. However, spatial variability was highly significant on PC1 ($F_{4, 91}$ = 7.258, p <0.001) and PC2 ($F_{4, 91}$ = 7.882, p <0.001), with scores on both components generally declining with progression downstream from site 1 to 5. Declining PC1 scores reflected a reduction in conductivity, low DO concentrations at site 1 and high water depths at site 5. PC2 scores decreased in response to increasing temperature with movement downstream and also reflected low velocities at site 5.

Hyporheic water

PCA ordination of hyporheic water data is presented both by month and by site (Figure 5.11). PC1 explained 47.9 % of the variance and PC2 a further 26.0 %, with



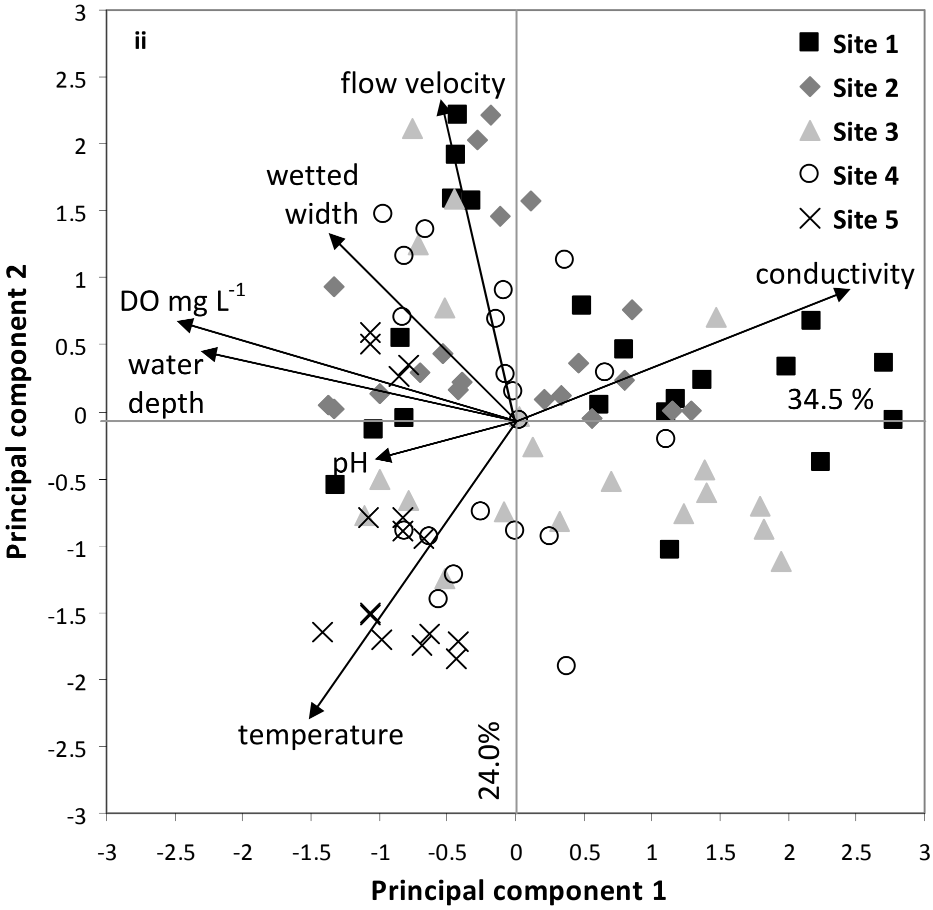


Figure 5.10: Principal components analysis ordination of surface water data: i) temporal variability; ii) spatial variability in environmental conditions.

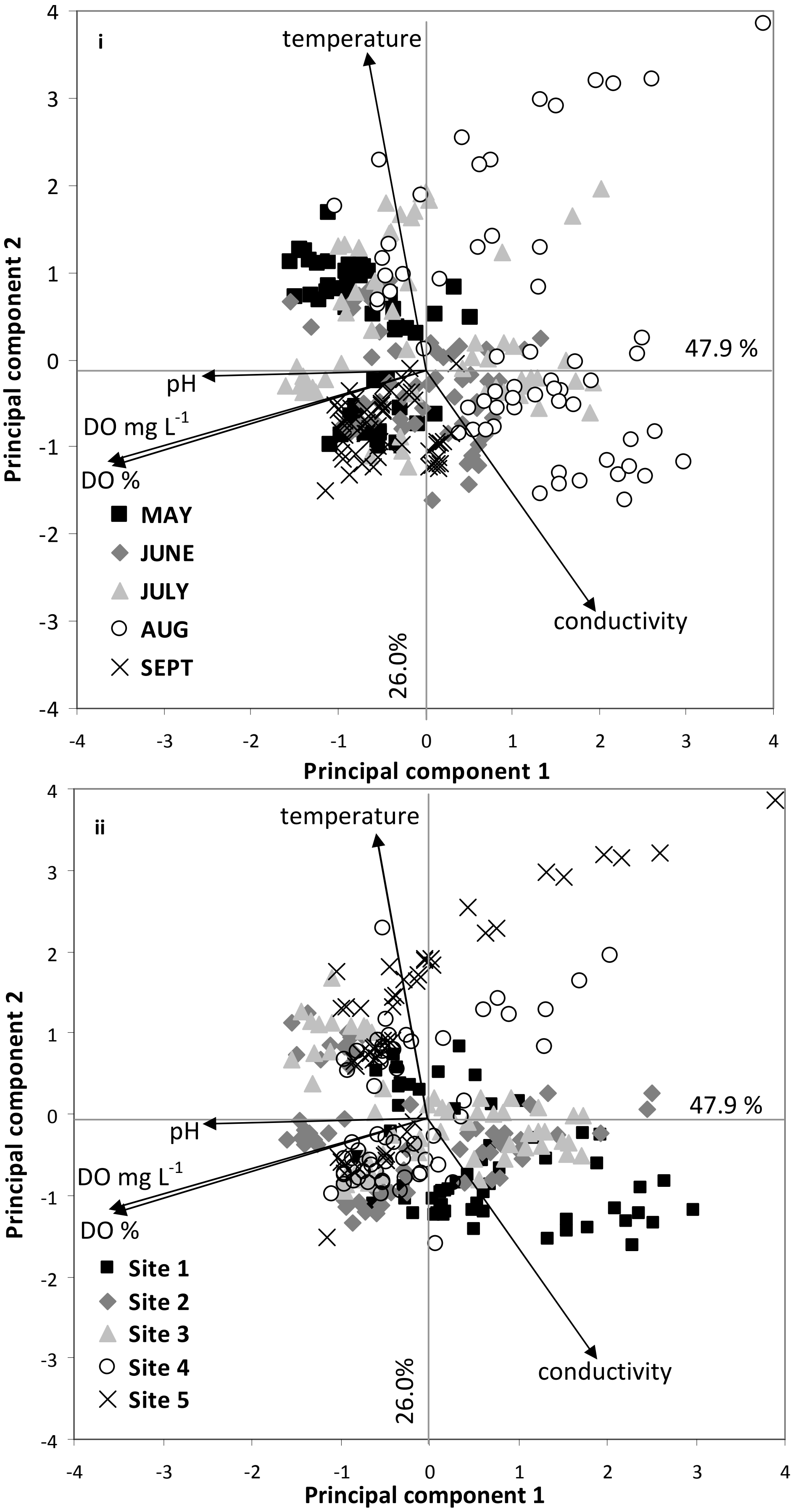


Figure 5.11: Principal components analysis of hyporheic water chemistry data: i) temporal variability; ii) spatial variability in environmental conditions.

both principal components having significant correlations with most environmental variables. In particular, PC1 was negatively correlated with both DO variables (PCC = -0.9, $p < 0.001$), and PC2 was positively correlated with temperature (PCC = 0.868, $p < 0.001$).

Considerable overlap was observed between samples from all months, with most samples plotting near the origin (Figure 5.11(i)). Temporal change was significant on both PC1 ($F_{4,\,283} = 48.462$, $p < 0.001$) and PC2 ($F_{4,\,283} = 18.794$, $p < 0.001$). PC1 scores were low in May and September, intermediate in June and July, and particularly high in August, reflecting changes in DO availability. Temporal change on PC2 reflected temperature variability, with scores being similar in May, July and August when temperatures were high, and mainly negative in September when temperatures were at their lowest. Samples with both the highest and lowest PC2 scores occurred in August, due to the highly variable temperatures in this month.

There was also considerable overlap in environmental conditions between sites, with samples from sites 2-4 clustering near the origin (Figure 5.11(ii)). Despite this, spatial change was significant on both PC1 ($F_{4,\,283} = 10.047$, $p < 0.001$) and PC2 ($F_{4,\,283} = 30.802$, $p < 0.001$). PC1 scores were particularly high at site 1 due to low DO values, and similar at all other sites. PC2 scores increased gradually with progression downstream, with scores being particularly low at site 1 and very high at site 5, reflecting a gradual decline in conductivity and an increase in temperature as groundwater dominance declined with progression downstream.

5.4.5 Environmental conditions at site 5 in 2009

Data was also collected during a second survey season between May and September 2009. Measurements were restricted to site 5, as this site had been selected for installation of hyporheic monitoring equipment (data not presented). This data is presented to allow comparison with patterns observed in 2008.

Surface hydrology

Discharge data is not available for 2009, but instream conditions suggested that flow recession was less pronounced than during 2008. Wetted width was comparable in all months, whilst depth varied little and always exceeded 2008 depths (Table 5.9). Submerged habitat availability was therefore similar and high in all months. Mean velocity was lowest in June and considerably higher in July ($F_{1.363, 4.090} = 10.335$, $p = 0.028$) but remained within the range recorded in 2008.

Table 5.9: Temporal change in hydrological and physicochemical measures of surface and hyporheic water at site 5, May to September 2009

	Surface/ Hyporheic	**May**	**June**	**July**	**August**	**Sept**	**Temporal change**
Hydrological variables							
Depth (cm)	Surface	52.1 ± 3.4	48.5 ± 2.6	56.5 ± 2.9	51.5 ± 2.9	49.5 ± 2.9	*
Mean velocity (m s^{-1})	Surface	0.07 ± 0.02	0.03 ± 0.01	0.09 ± 0.03	0.05 ± 0.01	0.04 ± 0.01	*
Width (m)	Surface	5.8 2 ± 0.1	5.71 ± 0.1	5.74 ± 0.1	5.68 ± 0.2	5.66 ± 0.2	ns
Water chemistry variables							
DO (mg L^{-1})	Surface	9.9 ± 0.08	8.7 ± 0.21	6.0 ± 0.16	7.9 ± 0.17	6.7 ± 0.07	**
	Hyporheic	7.3 ± 0.19	4.9 ± 0.43	4.7 ± 0.15	5.1 ± 0.27	5.5 ± 0.21	**
DO (% saturation)	Surface	102 ± 1.4	92.0 ± 1.9	67.3 ± 3.4	86.3 ± 1.4	84.8 ± 0.5	**
	Hyporheic	79.1 ± 1.6	62.5 ± 3.4	59.7 ± 1.2	65.1 ± 2.1	75.3 ± 1.6	**
Temperature (^{o}C)	Surface	10.3± 0.05	10.7± 0.05	9.6 ± 0.03	10.1 ±0.03	10.1 ±0.03	**
	Hyporheic	11.1 ±0.07	11.0 ±0.06	10.1 ±0.04	10.3 ±0.03	10.1 ±0.01	**
pH	Surface	8.2 ± 0.03	8.2 ± 0.03	8.0 ± 0.03	8.1 ± 0.04	8.1 ± 0.03	ns
	Hyporheic	8.2 ± 0	8.0 ± 0.03	8.0 ± 0.03	8.1 ± 0.01	8.2 ± 0.01	**
Conductivity (µS cm^{-1})	Surface	579 ± 1.5	612 ± 0.5	593 ± 1.3	621 ± 0.3	624 ± 0.8	**
	Hyporheic	586 ± 0.55	621 ± 0.89	603 ± 0.58	631 ± 0.86	632 ± 0.83	**
POC (mg L^{-1})	Surface	2.4 ± 0.4	16.5 ± 3.7	15.8 ± 14	12.5 ± 5.5	37.8 ± 20	ns
	Hyporheic	24 ± 4.0	37 ± 15	40 ± 13	35 ± 9	24 ± 6	ns
Fine sediment (mg L^{-1})	Surface	1.3 ± 0.4	36 ± 12	38 ± 37	24 ± 14	103 ± 53	ns
	Hyporheic	245 ± 109	529 ± 237	819 ± 370	506 ± 137	369 ± 207	ns

Values given as the mean ± 1 SE of all samples. In surface water, $n = 4$ and in hyporheic water $n = 12$. Temporal change analysed using one-way RM ANOVA, * indicates $p < 0.05$, ** indicates $p < 0.01$, ns indicates $p > 0.05$. Key – see Table 5.7.

Surface and hyporheic water chemistry

Differences between surface and hyporheic water chemistry reflect those reported in 2008. In both environments, water physicochemistry variables remained moderate throughout the study, with values comparable to those recorded in 2008 (Table 5.9). DO availability was low in July, with individual values as low as 5 mg L^{-1}/ 58 % in surface water and 3.1 mg L^{-1}/48 % in hyporheic water.

5.5 Spatiotemporal variability in the benthic invertebrate community

Considering the multi-site data collected in 2008, spatial and temporal variability in the composition of the invertebrate community inhabiting the benthic sediments is examined to identify changes in the abundance of taxa with the potential to increase the strength of biotic interactions (aim 1, objective 6), and to consider changes in community composition related to variation in hydrological or hydrologically-mediated parameters (aim 2, objectives 1 and 2).

Community description

A total of 35,018 individuals were recorded from 96 Surber samples. These individuals were drawn from ≥68 taxa, including 44 taxa identified to species level and 24 higher taxa that probably contained multiple representatives.

5.5.1 Detrended correspondence analysis

DCA was used to investigate spatial and temporal variability in benthic invertebrate community composition (Figure 5.12). Axis 1 explained 19.6 % of the variation and had highly significant (p <0.01) correlations with the abundance of *Gammarus pulex*, *Serratella ignita, Drusus annulatus* (pupae), and *Elmis aenea* and *Riolus* spp. larvae. Axis 2 explained a further 16.5 % of the variation and was significantly correlated with the abundance of *Leuctra* spp., *Agapetus fuscipes* (larvae), Oligochaeta and *E. aenea* and *Riolus* spp. larvae (Figure 5.12; Figure 5.13).

Temporal variability

Considerable overlap was observed between months (Figure 5.12(i)), but temporal change was significant on both axes. Axis 1 scores were stable from May to July, increased in August and peaked in September ($F_{2.201,\ 41.826}$ = 34.339, p = 0.001). Axis 2 scores were lower in May and September than in the intervening months ($F_{2.590,\ 49.214}$ = 26.802, p <0.001). A sample collected from site 3 following streambed drying in

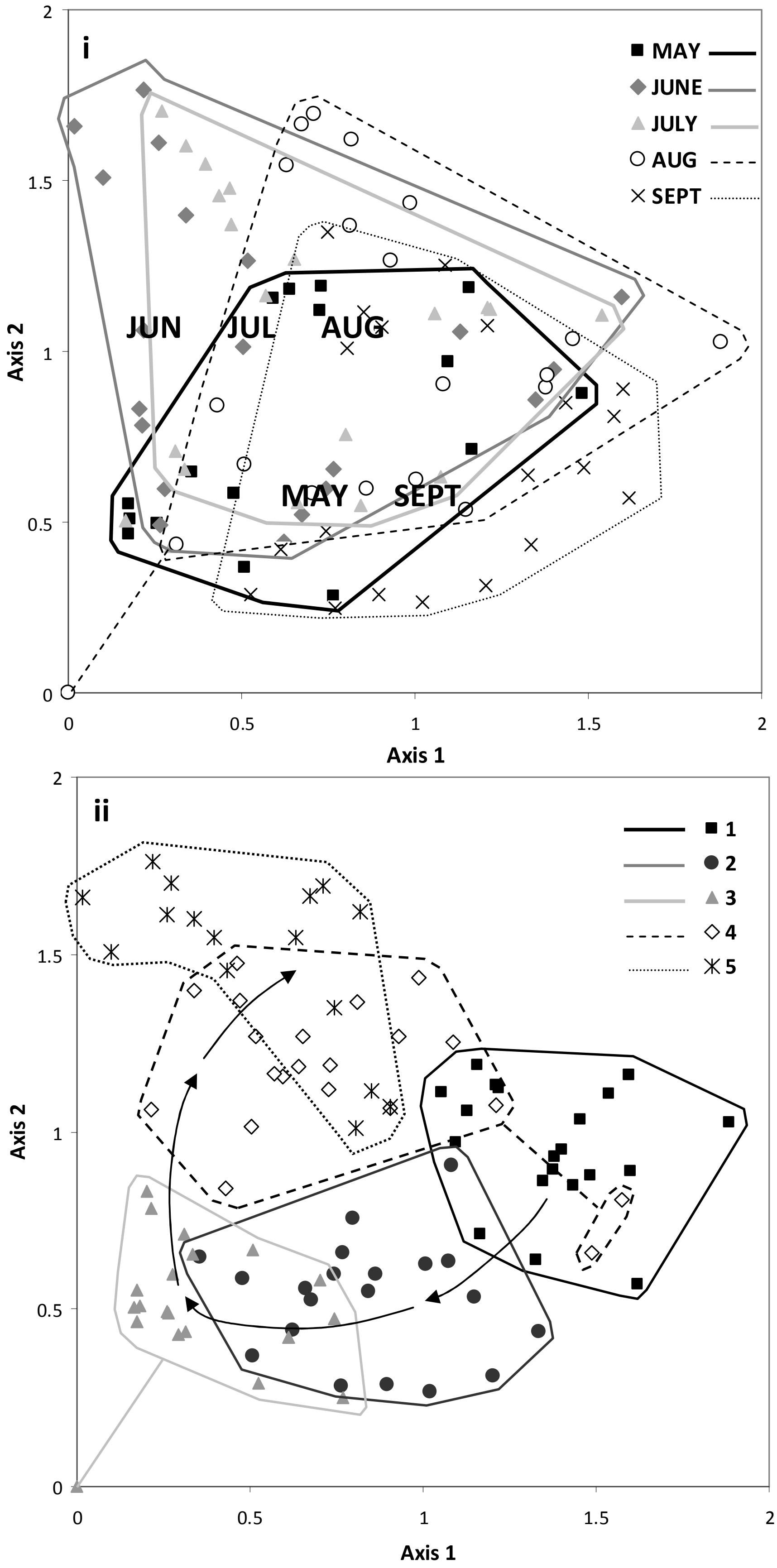


Figure 5.12: Detrended correspondence analysis sample plot of benthic community data: i) temporal variability; ii) spatial variability

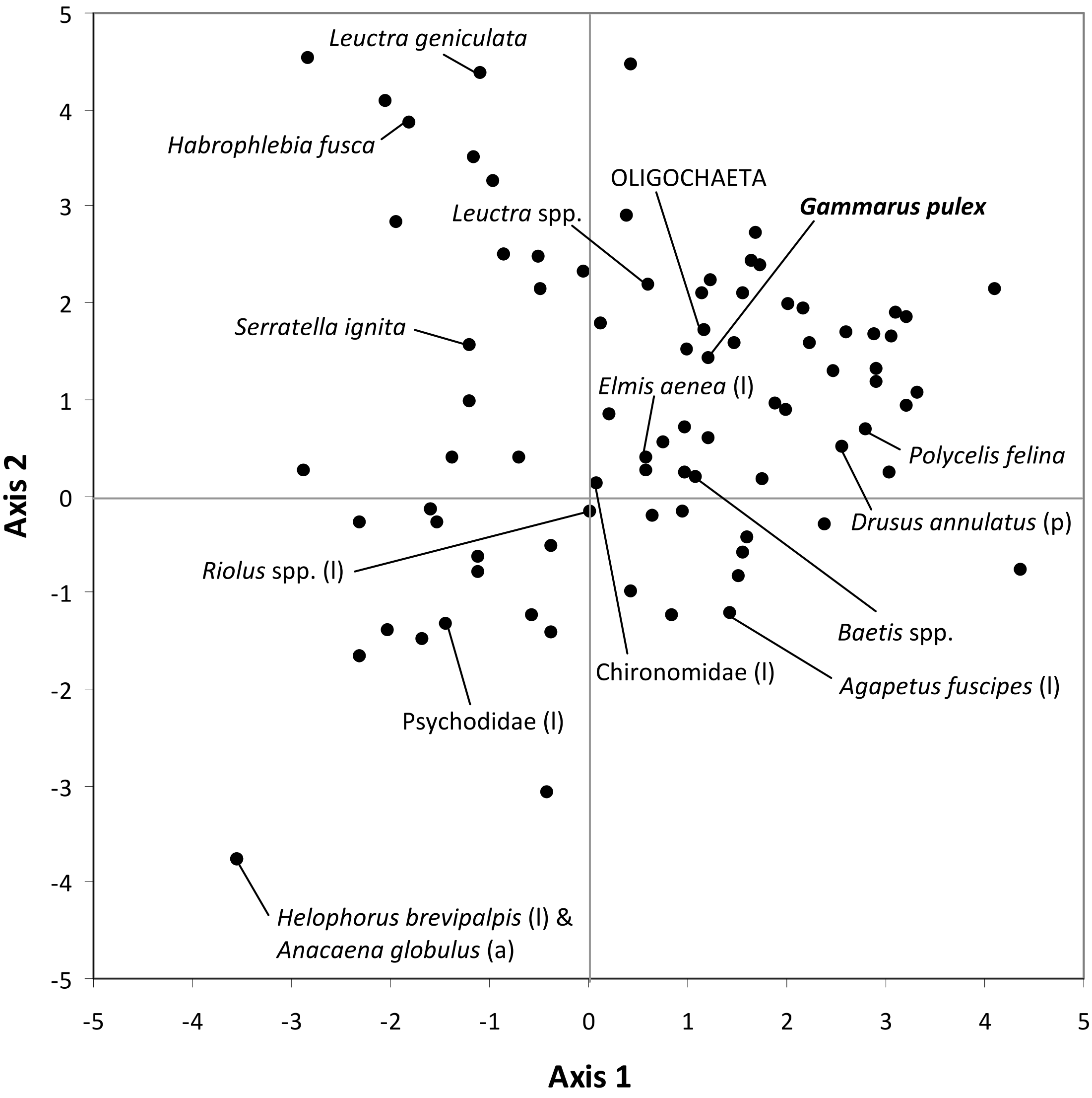


Figure 5.13: Detrended correspondence analysis species plot of benthic community data collected on the River Lathkill. Only common taxa (>1 % of all invertebrates) and notable outliers are indicated.

August plotted at the origin (Figure 5.12). Despite significant axis score correlations, taxa that did not experience significant temporal changes in abundance (e.g. *E. aenea* larvae) plotted near the centre of the species ordination (Figure 5.13). Positioning of other taxa partly reflected temporal changes in abundance, for example *Agapetus fuscipes* larvae were significantly more abundant in September that in other months and had high axis 1 and low axis 2 scores. Positioning of some taxa was not linked to their temporal occurrence, for example Psychodidae plotted in the negative quadrants of both axes despite high August abundance.

Spatial variability

A partial clockwise hysteresis effect occurred from upstream to downstream, with some overlap apparent between sites (Figure 5.12(ii)). Axis 1 scores were high at site 1, intermediate at sites 2 and 4 and low at sites 3 and 5 ($F_{4,\,15}$ = 61.268, p <0.001); axis 2 scores were high at site 5, intermediate at sites 1 and 4, and low at sites 2 and 3 ($F_{4,\,15}$ = 70.262, p <0.001). These combinations of axes scores resulted in each site forming a fairly distinct cluster (Figure 5.12(ii)) Ubiquitous taxa (Chironomidae, *Elmis aenea* larvae) plotted at the centre of the species ordination (Figure 5.14). Positioning of other taxa reflected associations with particular sites, for example *Leuctra geniculata* was largely restricted to site 5 and had a high axis 2 score, *Polycelis felina* occurred mainly at site 1 and scored highly on axis 1, whilst the Psychodidae were most common at site 3 and plotted in the negative quadrant of both axes (Figure 5.13).

5.5.2 Community metrics

Four metrics were calculated to characterise temporal change in benthic invertebrate community composition: total invertebrate abundance (TIA), taxon richness, the Berger-Parker dominance index and Simpson's Diversity Index (Section 4.9.1).

Total invertebrate abundance

TIA varied between 2540 0.1m^{-2} at site 3 in July (largely due to high chironomid abundance) and 31 0.1m^{-2} at site 4 in June. TIA differed between sites ($F_{4,\,15}$ = 3.523, p = 0.032), and was highest at site 1 and lowest at site 5, but there was no gradual decline with progression downstream (Table 5.11). Overall temporal change in TIA was of marginal significance ($F_{1.987,\,29.805}$ = 3.081, p = 0.061), with an increase between May and June, stability between June and August, and a sharp decline in September; *post-hoc* pairwise comparisons indicated that significant differences related only to low September values (Figure 5.14(i)). There was no significant interaction between TIA and site ($F_{4.991,\,19.966}$ = 1.240, p = 0.328), with the lowest abundances occurring in September at all sites except site 1 and peak abundances occurring between June and August (Figure 5.14(i)).

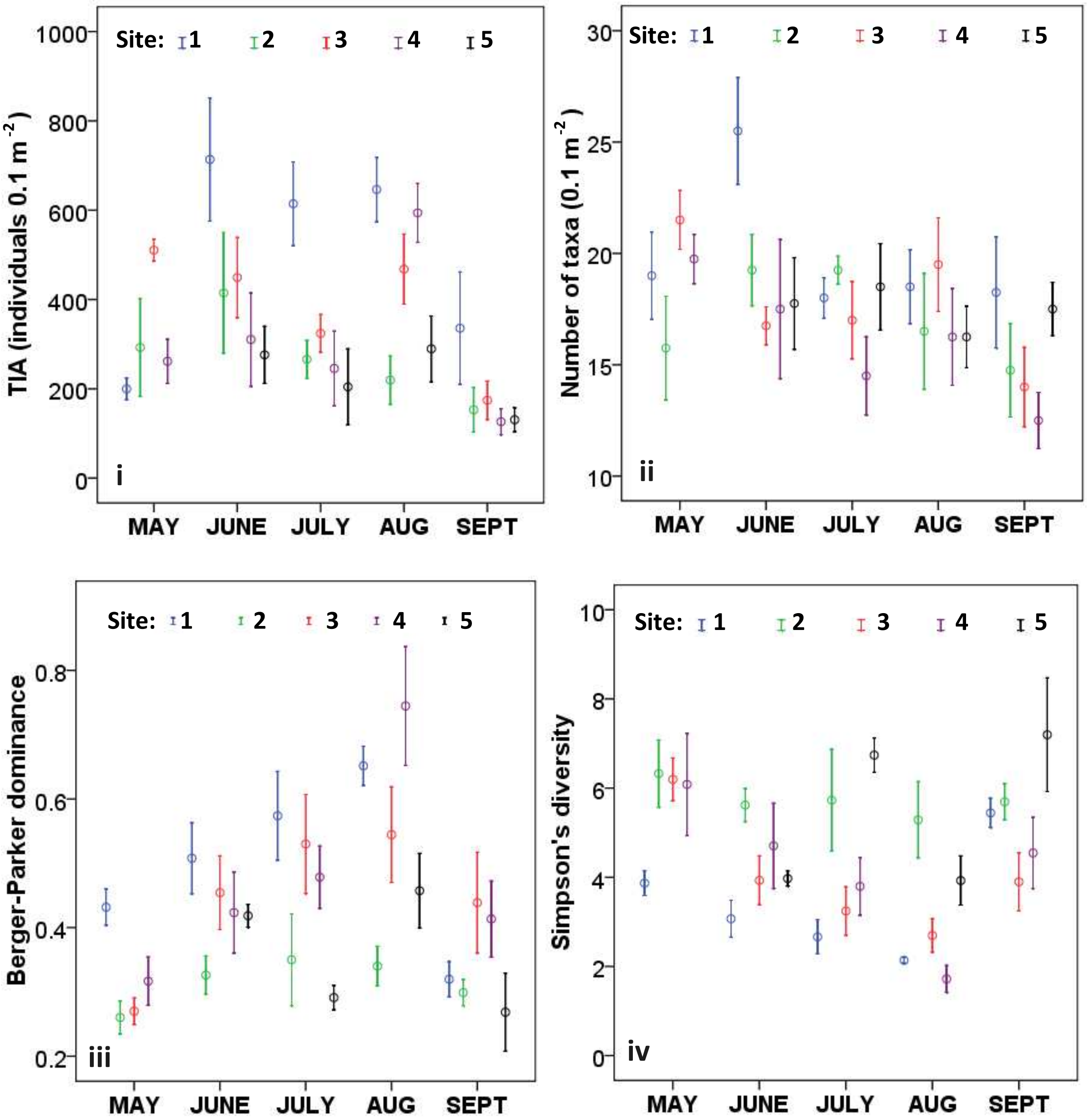


Figure 5.14: Mean ± 1 SE temporal change in benthic community metrics: i) total abundance (TIA; individuals 0.1m^{-2}; an outlier recorded at site 3 in July has been normalised to be in line with other samples); ii) number of taxa (taxa 0.1m^{-2}); iii) Berger-Parker dominance; iv) Simpson's diversity.

Number of taxa

The number of taxa per 0.1m^2 sample varied between 9 in samples from sites 2 and 4 in September up to 31 at site 1 in June. Mean taxon richness was highest at site 1 and lowest at site 4 ($F_{4,\,15}$ = 2.070, p = 0.136; Table 5.11). Considering all sites, taxon richness was highest in May and June, intermediate in July and August and lowest in September ($F_{4,\,60}$ = 4.585, p = 0.003; Table 5.10; Figure 5.14(ii)). The interaction with taxon richness was significant for flow permanence groups ($F_{4,\,56}$ = 4.359, p = 0.004) and of marginal significance for sites ($F_{12,\,48}$ = 1.914, p = 0.056). Peak abundance occurred in May at sites 3 and 4, in June at sites 1 and 2, and in July at site 5, and lowest richness occurring in July at site 1, August at site 5 and in September at sites 2, 3 and 4. However, temporal change was only significant at site 1, due to high richness in June ($F_{2.228,\,6.683}$ = 12.841, p = 0.005). Partial streambed drying promoted taxon richness in certain groups, for example one sample taken from an area of damp vegetation supported 16 beetle taxa, including two (*Anacaena globulus* adults and *Helophorus brevipalpis* larvae) not recorded elsewhere.

Table 5.10: Temporal change in benthic community metrics in the River Lathkill, May to September 2008

Variable	May	June	July	August	Sept	Temporal change
Total invertebrate abundance (0.1m^{-2})	316 ± 41	433 ± 56	438 ± 119	443 ± 47	184 ± 32	ns
Number of taxa	19.0 ± 0.9	19.4 ± 1.1	17.5 ± 0.7	17.4 ± 0.9	15.4 ± 0.9	**
Berger-Parker dominance	0.32 ± 0.02	0.43 ± 0.02	0.44 ± 0.03	0.55 ± 0.04	0.35 ± 0.03	**
Simpson's diversity	5.6 ± 0.4	4.3 ± 0.3	4.4 ± 0.5	3.2 ± 0.4	5.4 ± 0.4	**

All values presented as mean ± 1 SE, n = 16 in May and n = 20 from June onwards. Temporal change analysed using one-way RM ANOVA;** indicates p <0.01 and ns indicating p >0.05.

Table 5.11: Spatial differences in benthic community metrics at River Lathkill sites 1-5

Variable	Site 1	Site 2	Site 3	Site 4	Site 5	Spatial change
Total invertebrate abundance (0.1m^{-2})	502 ± 60	269 ± 40	492 ± 114	308 ± 46	225 ± 34	*
Number of taxa	19.9 ± 1.0	17.1 ± 0.9	17.7 ± 0.9	16.1 ± 1.0	17.5 ± 0.8	ns
Berger-Parker dominance	0.50 ± 0.03	0.32 ± 0.02	0.45 ± 0.03	0.48 ± 0.04	0.36 ± 0.03	**
Simpson's diversity	3.4 ± 0.3	5.7 ± 0.3	4.0 ± 0.3	4.2 ± 0.5	5.5 ± 0.5	**

All values presented as mean ± 1 SE, n = 20 at sites 1-4 and n = 16 at site 5. Spatial change analysed using two-way RM ANOVA, with ** indicating p <0.01 and ns indicating p >0.05.

Berger-Parker dominance and Simpson's diversity indices

Berger-Parker dominance varied between 0.19 at site 5 in September and 0.84 at site 4 in August. Dominance differed between sites ($F_{3,\,12}$ = 7.680, p = 0.004), being highest at site 1 and lowest at site 2 (Table 5.11). Mean dominance values increased each month between May and August then fell back to May levels in September ($F_{4,\,60}$ = 11.839, p <0.001; Table 5.10; Figure 5.14(iii)). The interaction between the dominance index and site was significant ($F_{12,\,48}$ = 2.195, p = 0.027) due to only minor variation in the significance of the overall pattern.

Simpson’s diversity ranged between 1.4 at site 4 in July up to 9.3 at site 5 in September and site 4 in May. Patterns of spatial and temporal change were the opposite of those reported for dominance. Spatially, diversity was higher at sites 2 and 5 compared with other sites ($F_{3,\,12}$ = 11.130, p <0.001; Table 5.11). Temporally, diversity was high in May, declined in June, remained stable in July, fell again in August then recovered in September ($F_{4,\,60}$ = 8.884, p <0.001; Table 5.10; Figure 5.14(iv)). There was no significant interaction between diversity and either spatial parameter.

5.5.3 Abundance of common taxa

Twelve taxa accounted for >1 % of all recorded individuals, and together these taxa accounted for >92 % of the total community (Table 5.12). One common taxon, larvae of the genus *Riolus* (probably *R. subviolaceus* (Coleoptera: Elmidae)) is nationally notable in terms of conservation status (Chadd and Extence, 2004).

Table 5.12: Occurrence of the common benthic invertebrates (>1 % total invertebrate abundance) in the River Lathkill

Taxon	Total no. of individuals	% of community	Cumulative % of community	Present in *x* % of samples
Gammarus pulex	13560	38.7	38.7	99.0
CHIRONOMIDAE (l)	6157	17.6	56.3	97.9
Polycelis felina	2337	6.7	63.0	58.3
OLIGOCHAETA	1712	4.9	67.9	95.8
Leuctra spp.	1598	4.6	72.4	82.3
Serratella ignita	1462	4.2	76.6	64.6
Baetis spp.	1453	4.1	80.8	77.1
Elmis aenea (l)	1372	3.9	84.7	99.0
Riolus spp. (l)	795	2.3	86.9	82.3
Drusus annulatus (p)	759	2.2	89.1	47.9
Agapetus fuscipes (l)	707	2.0	91.1	44.8
PSYCHODIDAE	460	1.3	92.4	30.2
Total	32372	92.4	92.4	-

Gammarus pulex

Gammarus pulex (Crustacea: Amphipoda) was the most abundant member of the benthos, accounting for 38.7 % TIA. This taxon was also widespread, occurring in all but one sample, at mean densities of 141 0.1m^{-2} and a peak density of 648 $0.1\ \text{m}^{-2}$. *G. pulex* abundance differed between sites ($F_{3,\,12}$ = 10.128, p = 0.001) due to particularly high abundances at site 1 (Table 5.14). Mean *G. pulex* abundance increased between May and June, remained stable in July then peaked in August before falling sharply in September ($F_{2.246,\ 33.686}$ = 8.530, p = 0.001; Table 5.13; Figure 5.15(i)). The interaction between *G. pulex* abundance and site was significant ($F_{12,\ 48}$ = 2.498, p = 0.012), and whilst the lowest abundances occurred in September at all sites, patterns of temporal change varied in preceding months. At site 1, abundance was relatively low in May and much higher in June, July and, in particular, August; at site 2, abundance was particularly high in June; at site 3, abundance increased to a peak in July; at site 4, the overall pattern was observed; and at site 5, abundance fell between June and July before peaking in August (Figure 5.15(i). These patterns were significant at sites 1 and 4.

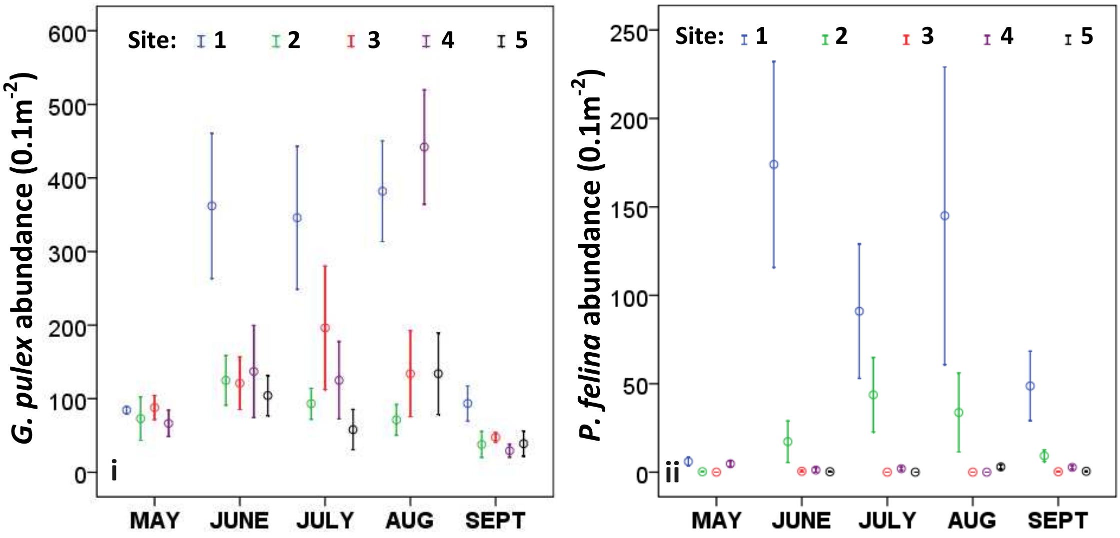


Figure 5.15: Mean ± 1 SE temporal change in benthic abundance of common taxa (individuals per 0.1 m^2 sample): i) *Gammarus pulex*; ii) *Polycelis felina*.

Chironomidae (larvae)

Species of chironomid (Diptera) larvae accounted for 17.6 % TIA and occurred in all but two samples. Whilst changes in chironomid abundance during the summer months may reflect seasonal emergence of adult life stages, they are considered briefly here due to their common occurrence. Chironomid abundance peaked at 1920 0.1 m^{-2} at site 3 in July; such values were atypical and mean abundance was 64 0.1 m^{-2}. Chironomid abundance was particularly high at site 3 and comparable at all other sites ($F_{4,\ 15}$ = 7.136, p = 0.002; Table 5.14). Chironomid abundance increased to a peak in July then declined sharply in August and was lowest in September; temporal change was not significant ($F_{1.676, 25.144} = 1.372$, $p = 0.269$; Table 5.13). The interaction between site and chironomid abundance was not significant.

Polycelis felina

Polycelis felina (Turbellaria: Tricladida) was the third most common taxon, accounting for 6.7 % TIA. *P. felina* was not widespread, occurring in 58.3 % of samples at densities of up to 392 0.1 m^{-2}. The taxon was largely restricted to site 1 (79.5 % of all individuals; $F_{3, 12} = 17.649$, $p = 0.013$ compared with all other sites) and was also common at site 2 (Table 5.14). *P. felina* abundance was very low May, increased >10-fold to a June peak, remained high in July and August, then fell in September ($F_{1.834, 27.506} = 3.452$, $p = 0.050$; Table 5.13; Figure 5.15(ii)). The interaction with *P. felina* abundance was significant for site ($F_{4.895, 19.582} = 4.215$, $p = 0.009$). At site 1, mean abundance increased sharply between May and June then intermediate values occurred in all later months; temporal change was not significant at this or any other site.

Oligochaeta

The class Oligochaeta comprised 4.9 % of the benthic community and occurring in 95.8 % of all samples. Population densities ranged from zero in four site 3 samples up to 120 0.1 m^{-2} at site 1 in July; mean abundance was 17.8 0.1 m^{-2}. Oligochaete densities were low at site 3 compared with other sites but spatial variability was not significant ($F_{3, 12} = 3.258$, $p = 0.060$). Mean densities peaked in June then declined slightly in all subsequent months ($F_{2.831, 42.471} = 1.795$, $p = 0.165$).

Other common taxa

Other common taxa (*Leuctra* spp., *Serratella ignita*, *Baetis* spp., *Drusus annulatus*, *Agapetus fuscipes*, *Elmis aenea* and *Riolus* spp.) belonged to the Insecta were not considered further due to potentially confounding effects of seasonal abundance changes (Williams and Feltmate, 1992; Elliott, 2006). Summary data is provided in Tables 5.13 and 5.14.

Table 5.13: Temporal change in the benthic abundance of common taxa in the River Lathkill, May to September 2008

	Mean abundance (0.1m^{-2})					**Temporal change**
	May	**June**	**July**	**August**	**Sept**	
Gammarus pulex	78 ± 9	170 ± 32	164 ± 35	233 ± 42	49 ± 8	**
CHIRONOMIDAE (l)	57 ± 15	62 ± 21	132 ± 14	37 ± 14	32 ± 9	ns
Polycelis felina	2.8 ± 1	38.7 ± 18	27.4 ± 11	36.4 ± 20	12.3 ± 6	*
OLIGOCHAETA	16.8 ± 3.7	24.8 ± 6.8	22.2 ± 6.4	15.1 ± 3.7	10.2 ± 1.4	ns
Leuctra spp.	2.3 ± 0.8	16.5 ± 4.5	21.3 ± 5.6	37.9 ± 12	2.5 ± 0.8	**
Serratella ignita	34.1 ± 8.7	36.0 ± 8.2	8.4 ± 1.6	1.3 ± 0.5	0.3 ± 0.1	**
Baetis spp.	46.6 ± 9.7	17.3 ± 4.1	4.5 ± 0.9	9.1 ± 3.1	4.95 ± 3.1	**
Elmis aenea (l)	17.3 ± 4.5	13.8 ± 2.4	13.7 ± 2.5	15.5 ± 2.2	11.8 ± 2.7	-
Riolus spp. (l)	20.1 ± 8.3	6.9 ± 1.8	7.3 ± 2.3	5.3 ± 2.3	4.3 ± 1.4	*
Agapetus fuscipes (l)	7.9 ± 4.0	11.1 ± 6.4	3.9 ± 2.1	2 ± 1.2	12.1 ± 4.2	**

All values presented as mean ± 1 SE, *n* = 16 in May and *n* = 20 from June onwards. Temporal change analysed using one-way RM ANOVA; * indicates *p* <0.05, ** indicates *p* <0.01, ns indicates *p* >0.05

Table 5.14: Spatial differences in the benthic abundance of common taxa at River Lathkill sites 1-5

	Mean abundance (0.1m^{-2})					**Spatial change**
	Site 1	**Site 2**	**Site 3**	**Site 4**	**Site 5**	
Gammarus pulex	254 ± 42	81 ± 12	117 ± 23	160 ± 39	84 ± 18	**
CHIRONOMIDAE (l)	31 ± 7	32 ± 11	209 ± 92	22 ± 5	18 ± 5	**
Polycelis felina	93 ± 24	21 ± 7	0.2 ± 0.1	2.2 ± 0.6	0.9 ± 0.4	**
OLIGOCHAETA	25 ± 7	20 ± 5	4 .1± 1.0	14.8 ± 2.1	27 ± 4.8	ns
Leuctra spp.	9.1 ± 3.0	10.2 ± 4.3	3.6 ± 1.1	37 ± 12	25 ± 6.1	*
Serratella ignita	1.1 ± 0.4	15 ± 6.3	24 ± 6.4	15 ± 3.7	23 ± 10	**
Baetis spp.	21 ± 5.3	14 ± 3.2	21 ± 6.1	17 ± 7.4	0.6 ± 0.2	*
Elmis aenea (l)	9.5 ± 1.1	17 ± 3.0	27 ± 3.3	6.8 ± 1.0	10.4 ± 1.6	*
Riolus spp. (l)	3.9 ± 1.2	9.4 ± 2.1	23.8 ± 6.5	1.7 ± 0.4	1.3 ± 0.2	*
Agapetus fuscipes (l)	4.3 ± 3.1	29 ± 6.0	1.3 ± 0.5	0.9 ± 0.6	0	**

Values presented as Mean ± 1 SE, *n* = 16 in May and *n* = 20 from June onwards. Spatial change analysed using two-way RM ANOVA; * indicates *p* <0.05, ** indicates *p* <0.01, ns indicates *p* >0.05

Notable rare taxa

Several other taxa of conservation interest also occurred at low abundance, with *Tinoides dives* (Trichoptera: Psychomyidae) also being nationally notable whilst *Mesovelia furcata* (Hemiptera: Mesoveliidae) is regionally notable (Chadd and Extence, 2004). In addition, the groundwater meiofauna species *Antrobathynella stammeri* (Syncarida: Bathynellidae), which is considered rare in the United Kingdom was observed in a hyporheic sample collected in June (details provided in Stubbington *et al.*, 2008).

5.6 Spatiotemporal variability in the hyporheic invertebrate community

To address aim 2 (objectives 1 and 2), the composition of the invertebrate assemblage inhabiting the hyporheic zone is examined, with particular reference to temporal changes in the occurrence of predominantly benthic taxa.

Community description

A total of 10,390 invertebrates were recorded in 288 hyporheic samples. Of these, 4565 individuals were recorded from a depth of 10 cm, 3031 from 20 cm and 2794 from 30 cm. Invertebrates belonged to ≥53 taxa, including 28 species and 27 higher taxa that probably contained multiple representatives.

5.6.1 Detrended correspondence analysis

Preliminary analyses indicated no significant differences in community composition at the three hyporheic depths, and all were therefore combined in subsequent analyses. DCA was used to examine both spatial and temporal variability in community composition (Figure 5.16; Figure 5.17). Axis 1 explained 11.4 % of the variation in the species data and had significant positive correlations with *Polycelis felina* and Cyclopoida abundance, and significant negative correlations with *Baetis* spp., Nematoda, Oligochaeta and Chironomidae (p <0.01). Axis 2 explained a further 7.6 % of the species variation and was positively correlated with Cyclopoida, Nematoda and Oligochaeta, and negatively correlated with *Gammarus pulex*, *Baetis* spp., *Serratella ignita* and Chironomidae (p <0.01).

Temporal variability

Samples from all months overlapped towards the centre of the ordination (Figure 5.16). Samples collected in May were particularly widely spaced, indicating highly variable community composition, whilst tighter clustering in both July and August indicated more homogenous community composition. Despite this variability, significant temporal change was observed on both axes 1 and 2. Axis 1 scores increased gradually to an August peak then fell in September ($F_{2.704,\ 146.626} = 10.550$, p <0.001), whilst axis 2 scores declined between May and June, increased moderately in July and August then peaked in September ($F_{3.377, 151.960} = 6.478$, $p < 0.001$; Figure 5.16).

Positioning of some taxa on the species ordination was related to their temporal occurrence (Figure 5.18). The Ostracoda, for example, were most abundant in August and September and plotted in the positive quadrant of both axes, whilst the June peak in *S. ignita* abundance is reflected by a low axis 2 score. In other cases, relationships were less apparent, for example the Nematoda had a particularly high score on axis 2 despite being uncommon in September; such results suggested the potential importance of spatial variability (Figure 5.18).

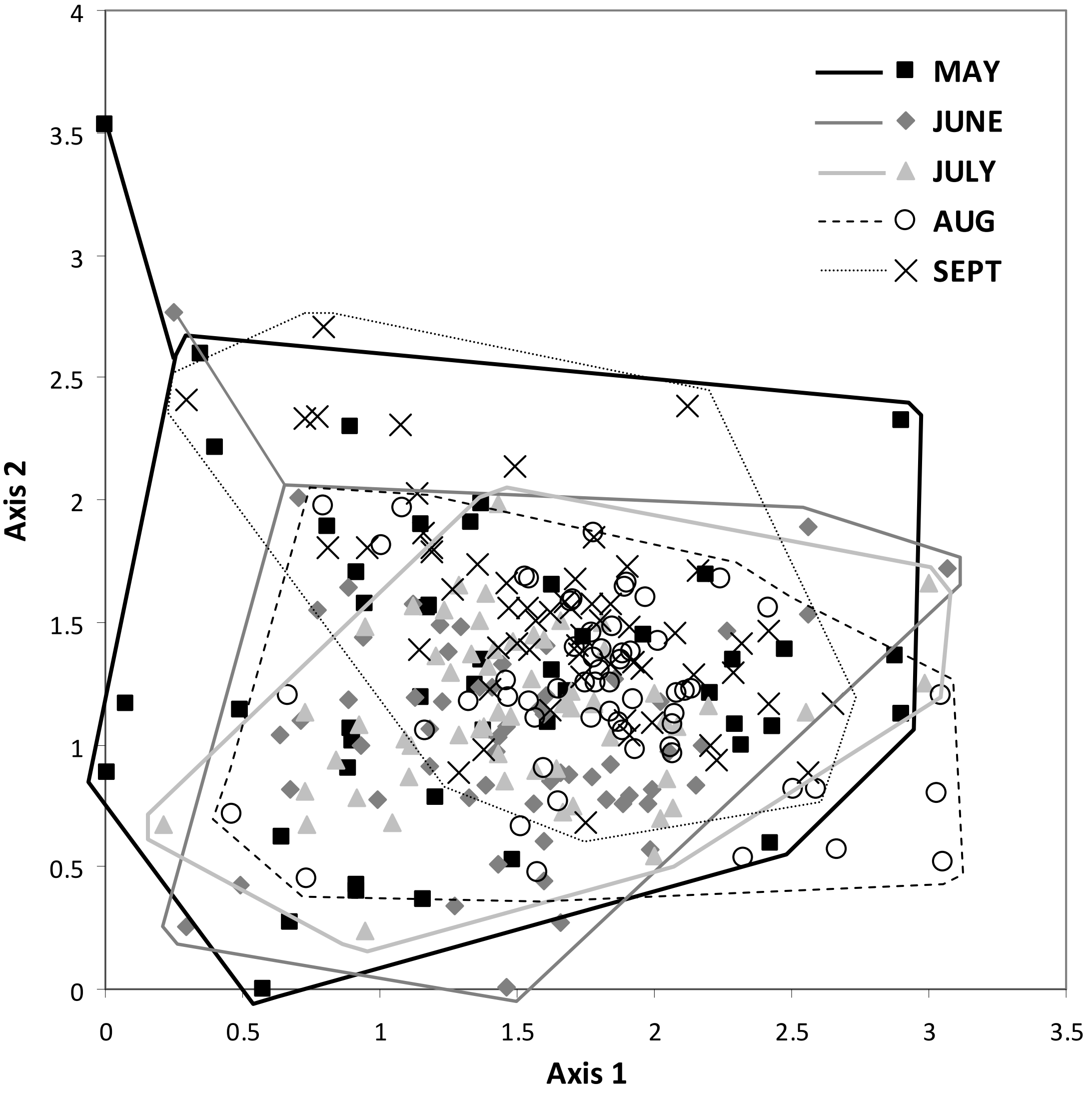


Figure 5.16: Detrended correspondence analysis sample plot of hyporheic community data, indicating temporal variability between May and September 2008.

Spatial variability

The DCA sample plot (Figure 5.17) indicated that the site 1 community was relatively distinct, whilst there was almost complete overlap between sites 2-5. Site 5 samples formed a distinct cluster towards the centre of axis 1, indicating homogenous community composition, whilst site 3 samples spanned the length of axis 2, indicating community variability. Axis 1 scores varied between sites due to site 1 scores being particularly high ($F_{4,\,53}$ = 25.875, p <0.001). Axis 2 scores were comparable at all sites ($F_{4,\,53}$ = 0.696, p = 0.598).

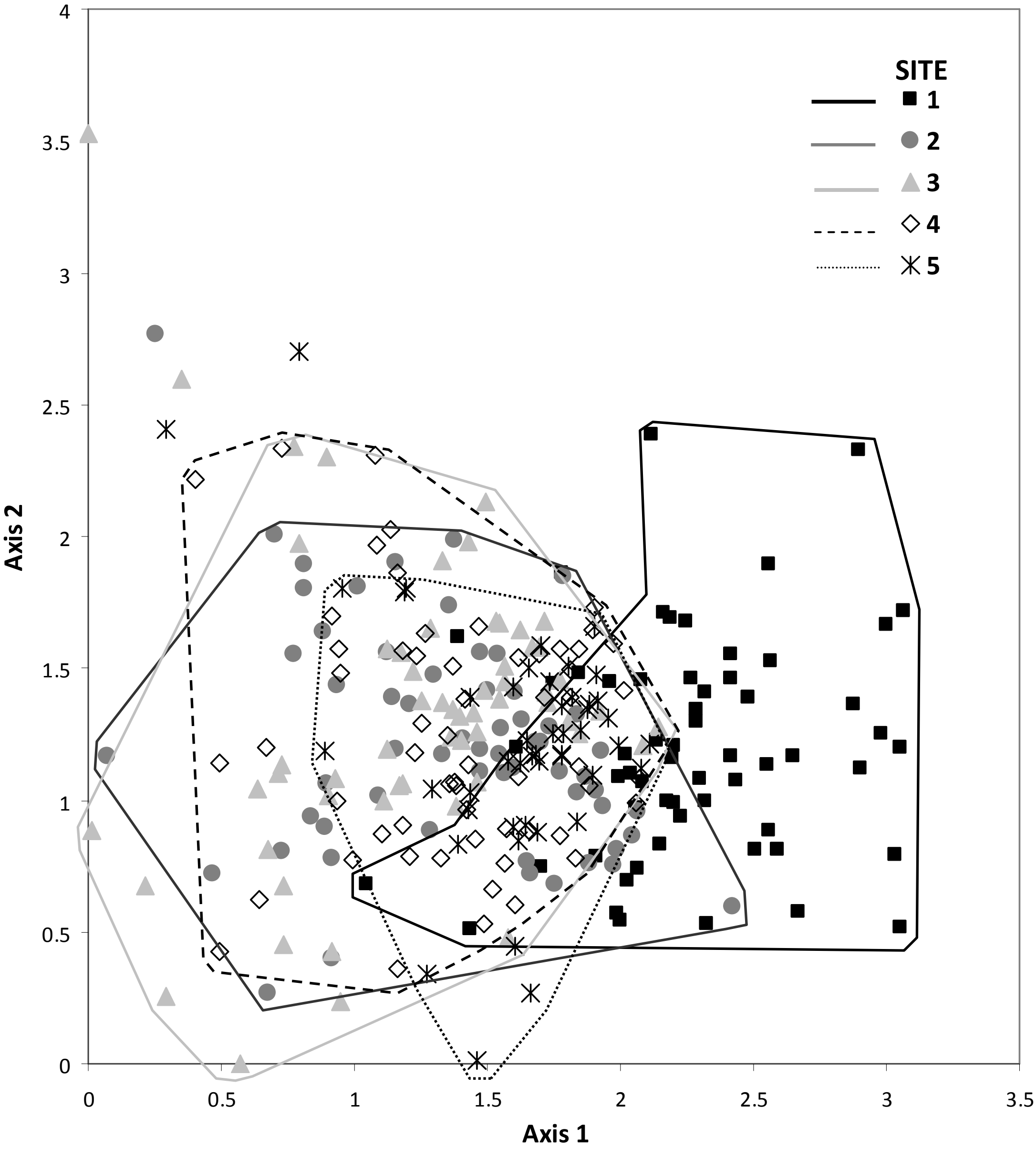


Figure 5.17: Detrended correspondence analysis sample plot of hyporheic community data, indicating site-specific differences.

Positioning of many taxa on the species plot could be related to spatial occurrence (Figure 5.18). *P. felina*, for example, was particularly abundant at site 1 and had a high axis 1 score, whilst high abundance of *Baetis* spp. at site 3 and the paucity of this taxon at site 1 was reflected by a negative axis 1 score. Several taxa (*G. pulex*, *S. ignita*, *Leuctra* spp.) were particularly abundant at site 5 and plotted near the centre of the ordination (Figure 5.18).

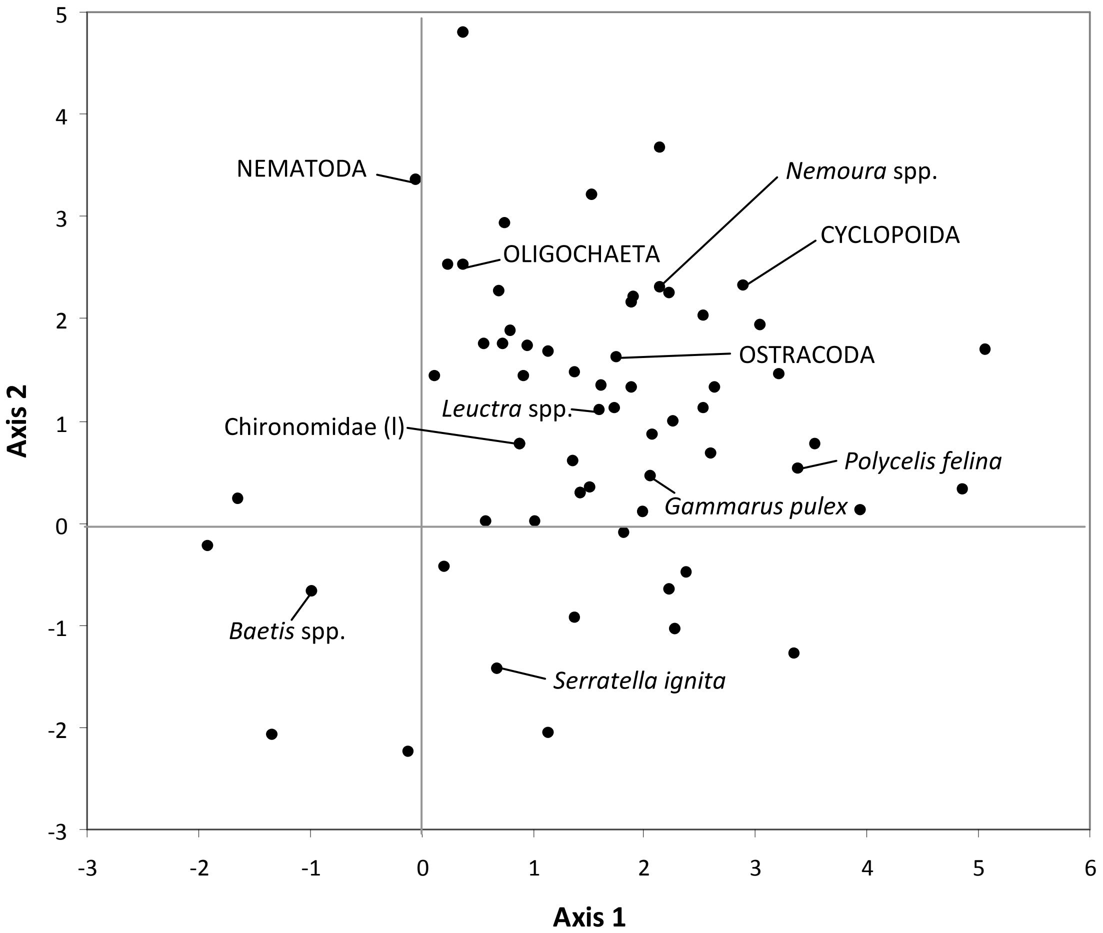


Figure 5.18: Detrended correspondence analysis species plot of hyporheic community data. Only common taxa (>1 % of all invertebrates) are indicated.

5.6.2 Community metrics

Total invertebrate abundance

Hyporheic TIA peaked at 260 6 L^{-1} in a sample collected from site 5 in August, whilst one sample taken at site 4 in September contained no invertebrates. TIA was higher at 10 cm (44.8 ± 3.6 6 L^{-1}) compared with 20 cm (23.6 ± 2.2 6 L^{-1}) and 30 cm (25.9 ± 2.7 $0.1m^{-2}$; p = 0.001), but there was no significant interaction between hyporheic depth and TIA ($F_{8, 180}$ = 1.316, p = 0.246). TIA was lowest at site 2 and particularly high at site 5 ($F_{4, 55}$ = 3.500, p = 0.013; Table 5.16). Overall, TIA increased steadily between May and August then declined back to June levels in September ($F_{4, 188}$ = 11.317, p <0.001; Figure 5.19(i); Table 5.15). There was no significant interaction between TIA and site ($F_{12, 176}$ = 1.527, p = 0.118).

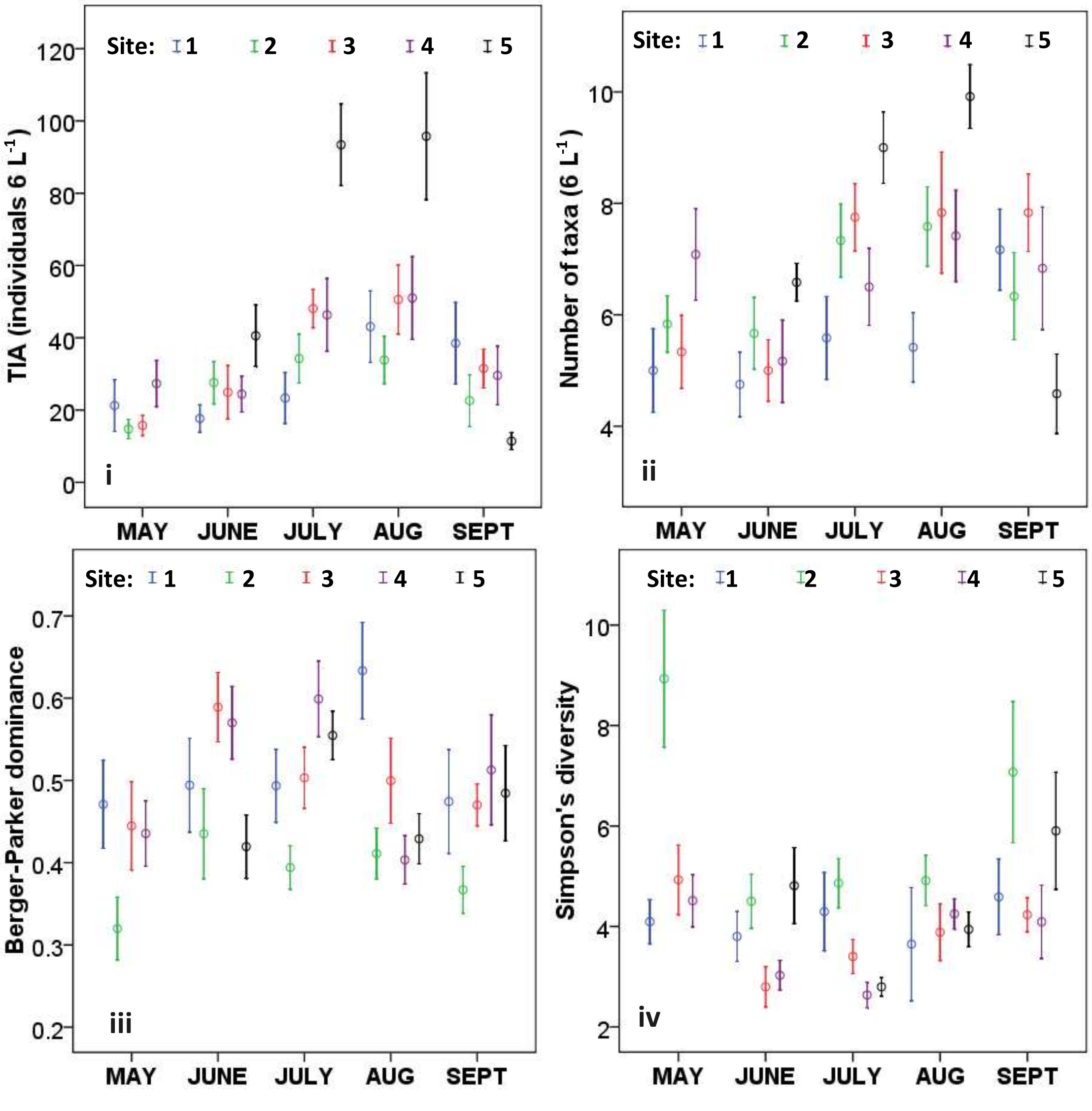


Figure 5.19: Mean ± 1 SE temporal change in hyporheic community metrics: i) total invertebrate abundance (TIA; individuals 6 L^{-1}); ii) number of taxa (taxa 6 L^{-1}); iii) Berger-Parker dominance; iv) Simpson's diversity.

Taxon richness

Taxon richness was higher at 10 cm (7.7 ± 0.3 taxa 6 L^{-1}) than at 20 cm (5.8 ± 0.3 taxa 6 L^{-1}) and 30 cm (5.7 ± 0.3 taxa 6 L^{-1}; $F_{2, 45}$ = 8.957, p = 0.001), but the interaction with hyporheic depth was not significant ($F_{8, 180}$ = 0.489, p = 0.863). Taxon richness was comparable at all sites ($F_{4, 55}$ = 1.647, p = 0.176; Table 5.16). Temporally, taxon richness was lowest in June, increased in July, peaked in August then declined moderately in September ($F_{4, 188}$ = 6.850, p <0.001; Table 5.15; Figure 5.19(ii)). Peak taxon richness, of 17 taxa (including multiple Crustacea, Plecoptera, Coleoptera and Diptera) occurred in a sample collected below an area of dried streambed at site 3 in August. The interaction with taxon richness was not significant for site ($F_{12, 176}$ = 1.470, p = 0.139), although at site 1 taxon richness was higher in September than in preceding months (Figure 5.19(ii)).

Table 5.15: Temporal change in hyporheic community metrics in the River Lathkill, May to September 2008

Variable	May	June	July	August	Sept	Temporal change
Total abundance (individuals 6 L^{-1})	19.8 ± 2.7	27.0 ± 2.9	49.1 ± 4.8	54.9 ± 5.7	26.7 ± 3.4	**
Taxon richness (taxa 6 L^{-1})	5.8 ± 0.4	5.4 ± 0.3	7.2 ± 0.3	7.6 ± 0.4	6.6 ± 0.4	**
Berger-Parker dominance	0.42±0.02	0.50±0.02	0.51±0.02	0.48±0.02	0.46±0.02	*
Simpson's diversity	5.6 ± 0.5	3.8 ± 0.2	3.6 ± 0.2	4.1 ± 0.3	5.2 ± 0.4	**

Values presented as mean ± 1 SE. Significance of temporal change determined using one-way RM ANOVA; * indicates p <0.05, ** indicates p <0.01

Table 5.16: Spatial differences in hyporheic community metrics at River Lathkill sites 1-4

Variable	Site 1	Site 2	Site 3	Site 4	Site 5	Spatial change
Total abundance (individuals 6 L^{-1})	29 ± 3.8	27 ± 2.8	34 ± 3.3	36 ± 3.9	60 ± 7.6	*
Taxon richness (taxa 6 L^{-1})	5.6 ± 0.3	6.8 ± 0.4	6.6 ± 0.4	6.6 ± 0.4	7.5 ± 0.4	ns
Berger-Parker dominance	0.5 ± 0.03	0.4 ± 0.02	0.5 ± 0.02	0.5 ± 0.02	0.5 ± 0.02	*
Simpson's diversity	4.1 ± 0.3	6.1 ± 0.5	3.8 ± 0.2	3.7 ± 0.2	4.4 ± 0.4	*

Values presented as mean ± 1 SE. Significance of spatial change determined using two-way RM ANOVA; * indicates p <0.05, ns indicates p >0.05

Berger Parker dominance and Simpson's diversity index

Berger-Parker dominance varied between 1 (complete dominance) in a sample containing only one individual, and 0.14 in a sample from site 1 in June comprising single individuals from seven taxa. Dominance was lower at 10 cm (0.42 ± 0.02) than at 20 and 30 cm (both 0.50 ± 0.02; $F_{2, 45}$ = 3.447, p = 0.040); the interaction between dominance and depth was not significant ($F_{8, 180}$ = 0.323, p = 0.957). Dominance was lower at site 2 than at other sites ($F_{4, 55}$ = 3.641, p = 0.011; Table 5.16). Overall, dominance was lowest in May, peaked in June then declined slightly in subsequent months ($F_{4, 188}$ = 3.325, p = 0.012; Table 5.15; Figure 5.19(iii)). The interaction with dominance was significant for site ($F_{12, 176}$ = 1.821, p = 0.048), with the overall pattern reflecting conditions at sites 2 and 3. At site 1, dominance was highest in August and comparable in all other months; at site 4, dominance peaked in July and was lowest in August; dominance also peaked in July at site 5 (Figure 5.19(iii)).

The sample containing a single invertebrate had the lowest Simpson's diversity value possible (1), whilst diversity was particularly high (16.5) in a sample taken from site 2 in May. Diversity was comparable at all hyporheic depths. Diversity was high at site 2 and similar at all other sites ($F_{4, 55}$ = 3.380, p = 0.015; Table 5.16). Mean diversity peaked in May, was lowest in June/July then increased moderately in August and September ($F_{2.747, 129.094}$ = 6.306, p = 0.001; Table 5.15; Figure 5.19(iv)). The interaction with diversity was not significant for site ($F_{7.717, 113.184}$ = 1.507, p = 0.165), although diversity was similar in all months at site 1, and was lowest in July then increased to a peak in September at site 5.

5.6.3 Abundance of common taxa

Preliminary analysis of DCA axis scores indicated that community composition was comparable at all hyporheic depths, and all were therefore combined in the subsequent analysis of community composition.

Eleven taxa accounted for >1 % TIA and these taxa collectively comprised 93.6 % of the hyporheos (Table 5.18). Of these, the Ostracoda, Cyclopoida, Oligochaeta and Nematoda can be classified as 'permanent hyporheos' (*sensu* Williams and Hynes, 1974) capable of completing their lifecycle in the hyporheic zone. Of these, Ostracoda, Cyclopoida and Nematoda are all meiofauna which may not have been sampled consistently and are therefore not considered in detail. Seven taxa were 'occasional hyporheos', i.e. predominantly benthic organisms that may inhabit the hyporheic zone for part of their lifecycle. The occasional hyporheos can be further subdivided into insect taxa (i.e. Chironomidae, *Leuctra*, *Nemoura*, *Baetis*, *Serratella*

Table 5.17: Occurrence of common invertebrates (>1 % total invertebrate abundance) in the hyporheic zone of the River Lathkill

Taxon	Total no. of individuals	% of community	Cumulative % of community	Present in *x* % of samples
OSTRACODA	2395	23.1	23.1	67.9
Chironomidae (l)	2282	22.0	45.0	89.2
Gammarus pulex	1506	14.5	59.5	71.4
Polycelis felina	1089	10.5	70.0	49.5
Leuctra spp.	591	5.7	75.7	40.1
CYCLOPOIDA	563	5.4	81.1	50.9
OLIGOCHAETA	562	5.4	86.5	49.1
Nemoura spp.	340	3.3	89.8	28.2
Serratella ignita	153	1.5	91.3	13.6
NEMATODA	129	1.2	92.5	27.2
Baetis spp.	113	1.1	93.6	18.1
Total	9723	93.6	93.6	-

Table 5.18: Temporal change in the abundance of common taxa in the hyporheic zone of the River Lathkill, May to September 2008

	Mean abundance (6 L^{-1})					Temporal change
	May	**June**	**July**	**August**	**Sept**	
OSTRACODA	1.9 ± 0.5	4.6 ± 1.3	14.2 ± 3.0	12.7 ± 3.1	6.9 ± 1.3	**
Chironomidae (l)	4.7 ± 1.0	7.9 ± 1.2	14.0 ± 2.0	8.2 ± 1.2	4.3 ± 0.8	**
Gammarus pulex	3.0 ± 0.7	3.3 ± 0.6	7.1 ± 1.3	10.0 ± 1.7	2.5 ± 0.6	**
Polycelis felina	2.1 ± 0.6	1.6 ± 0.4	2.7 ± 0.9	7.9 ± 2.4	4.3 ± 2.2	**
Leuctra spp.	0.7 ± 0.2	2.5 ± 0.6	2.8 ± 0.6	3.6 ± 0.9	0.5 ± 0.2	**
CYCLOPOIDA	1.7 ± 0.5	1.0 ± 0.4	1.8 ± 0.4	3.4 ± 0.5	1.8 ± 0.3	**
OLIGOCHAETA	2.5 ± 0.8	2.5 ± 0.9	1.8 ± 0.4	1.1 ± 0.2	2.0 ± 0.6	ns
Nemoura spp.	0	0	0.5 ± 0.1	4.0 ± 0.9	1.2 ± 0.2	**
Serratella ignita	0.6 ± 0.14	1.6 ± 0.79	0.4 ± 0.12	0.07±0.07	0	**
NEMATODA	0.9 ± 0.20	0.2 ± 0.06	0.6 ± 0.12	0.3 ± 0.09	0.3 ± 0.09	**
Baetis spp.	0.5 ± 0.13	0.4 ± 0.15	0.9 ± 0.23	0.3 ± 0.09	0	**

Values presented as mean ± 1 SE, *n* = 48 in May and *n* = 60 from June onwards. Temporal change analysed using one-way RM ANOVA; ** indicates *p* <0.01, ns indicates *p* >0.05

Table 5.19: Spatial differences in the abundance of common taxa in the hyporheic zone of River Lathkill sites 1-5

	Mean abundance (6 L^{-1})					Spatial change
	Site 1	**Site 2**	**Site 3**	**Site 4**	**Site 5**	
OSTRACODA	1.8 ± 0.4	2.8 ± 0.8	8.2 ± 1.7	5.4 ± 1.1	27 ± 4.4	**
Chironomidae (l)	2.7 ± 0.4	5.9 ± 0.7	10 ± 1.4	14 ± 2.1	6.7 ± 1.1	**
Gammarus pulex	3.9 ± 0.8	4.9 ± 0.9	3.4 ± 0.7	4.5 ± 1.1	10.6 ± 1.9	**
Polycelis felina	12 ± 3.0	3.6 ± 0.7	0.2 ± 0.1	1.5 ± 0.5	0.8 ± 0.3	**
Leuctra spp.	0.8 ± 0.2	1.9 ± 0.5	1.4 ± 0.4	2.8 ± 0.7	3.7 ± 1.0	ns
CYCLOPOIDA	3.9 ± 0.6	0.3 ± 0.1	2.5 ± 0.5	1.1 ± 0.3	2 ± 0.3	**
OLIGOCHAETA	1.1 ± 0.7	3.8 ± 0.9	2.3 ± 0.6	1.6 ± 0.3	0.8 ± 0.2	ns
Nemoura spp.	0.4 ± 0.2	0.4 ± 0.1	0.8 ± 0.3	1.7 ± 0.6	2.9 ± 0.9	**
Serratella ignita	0.03 ± 0.02	0.2 ± 0.08	0.13 ± 0.06	0.3 ± 0.1	2.3 ± 1.0	*
NEMATODA	0.02 ± 0.02	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	**
Baetis spp.	0.05 ± 0.04	0.5 ± 0.14	0.9 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	**

Values presented as mean ± 1 SE, *n* = 60 at sites 1-4 and *n* = 48 at site 5. Spatial differences analysed using two-way RM ANOVA; * indicates *p* <0.05, ** indicates *p* <0.01, ns indicates *p* >0.05

ignita) that emerge as adults during summer, and non-insect taxa (i.e. *Gammarus pulex*, *Polycelis felina*) that have no terrestrial life stage. Seasonal changes in the abundance of Insecta may confound attempts to relate their abundance to changes in environmental conditions, and they are therefore not examined in detail.

Gammarus pulex

G. pulex was the most abundant non-insect species of occasional hyporheos, comprising 14.5 % TIA (Table 5.17) and occurring at densities of ≤70 6 L^{-1}. The species was also widespread, occurring in 71.4 % of samples. Abundances were comparable at sites 1-4, with significant differences between sites reflecting high densities at site 5 ($F_{4, 55}$ = 5.004, p = 0.002; Table 5.19).

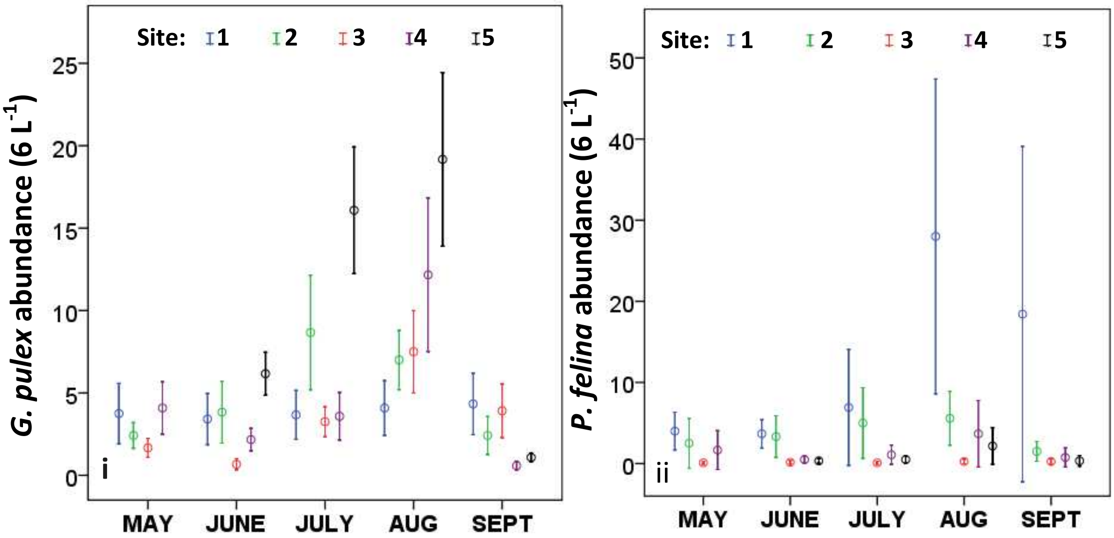


Figure 5.20: Mean ± 1 SE temporal change hyporheic abundance of benthic taxa (individuals 6 L^{-1}): i) *Gammarus pulex*; ii) *Polycelis felina*.

G. pulex abundance was low in May/June then increased threefold to a peak in August before falling sharply in September ($F_{4, 188}$ = 6.290, p <0.001; Figure 5.20(i); Table 5.18). The interaction with *G. pulex* abundance was significant for site when site 5 was included in an analysis of June to September data ($F_{12, 165}$ = 3.245, p <0.001). At site 1, *G. pulex* abundance was similarly low in all months. At site 2, abundance peaked in July, remained high in August then declined in September ($F_{1.871, 20.579}$ = 3.614, p = 0.048). At site 3, abundance was low in May/June then increased to an August peak ($F_{1.901, 20.915}$ = 3.728, p = 0.043). At site 4, *G. pulex* abundance was similarly low between May and July, increased threefold in August then declined to the lowest levels recorded in September ($F_{1.393, 15.323} = 3.511$, $p = 0.069$). At site 5, abundance increased threefold between June and August then declined dramatically in September ($F_{1.601, 17.608} = 8.751$, $p = 0.004$). In summary, at sites 3-5, *G. pulex* abundance increased threefold to a peak in August, this increase was less pronounced at site 2 and was not observed at site 1 (Figure 5.20(i)).

Polycelis felina

P. felina was the second of the two common non-insect members of the occasional hyporheos, accounting for 10.5 % TIA (Table 5.17). *P. felina* occurred in 49.5 % of samples at densities of ≤130 6 L^{-1}. The species was more abundant at perennial sites compared with intermittent sites ($F_{1, 46} = 27.029$, $p < 0.001$) and was more abundant at site 1 than at any other site (*post-hoc* paired *t*-tests, $p \leq 0.028$; Table 5.19). *A*bundance was stable from May to July, increased threefold to an August peak then declined moderately in September ($F_{2.661, 125.078} = 5.371$, $p = 0.002$; Table 5.18; Figure 5.20(ii)). The interaction with *P. felina* was significant for site ($F_{10.897, 149.833} = 2.290$, $p = 0.013$). At sites 1, 2 and 5, abundance increased to an August peak then fell in September ($p \leq 0.051$); few *P. felina* were recorded at sites 3 and 4 and temporal changes were not significant.

Cyclopoida

Microcrustacea may not have been sampled consistently due to their small size, and are therefore not considered in detail. However, it is worth noting that the Cyclopoida (Crustacea: Copepoda) were particularly abundant at site 1 ($F_{3, 44} = 13.084$, $p < 0.001$; Table 5.19), and temporally, were most abundant in August ($F_{4, 188} = 8.744$, $p < 0.001$; Table 5.18).

Oligochaeta

The Oligochaeta comprised 5.4 % TIA, occurring in 49.1 % of samples at densities of ≤46 6 L^{-1} (Table 5.17). Oligochaetes were most abundant at site 2 and rare at site 5 ($F_{4, 55} = 7.565$, $p < 0.001$; Table 5.19). Overall, oligochaete abundance did not change significantly between May and September (Table 5.18), and the interaction with site was not significant.

5.7 Benthic invertebrate use of the hyporheic zone

To determine the extent to which the hyporheic zone was used by benthic invertebrates, the proportion of the total (benthic + hyporheic) community within the hyporheic zone (i.e. the *hyporheic proportion*) is examined (aim 2, objective 3; also see Section 4.9.6). In addition to total invertebrate abundance, three predominantly benthic, non-insect taxa (*Gammarus pulex, Polycelis felina* and the Oligochaeta) were sufficiently common to warrant individual investigation of spatial and temporal variability in their use of the hyporheic sediments.

Total invertebrate abundance

The proportion of the total (benthic + hyporheic) invertebrate population inhabiting the hyporheic zone varied between 0.18 ± 0.03 at site 1 and 0.42 ± 0.05 at site 5 ($F_{4, 15} = 4.166$, $p = 0.018$). The hyporheic proportion was low in May and June and higher in all subsequent months ($F_{3, 45} = 3.147$, $p = 0.034$; Figure 5.21(i); Table 5.20), and the interaction with site was significant ($F_{12, 45} = 2.089$, $p = 0.037$). Temporal change was not significant at sites 1-4, with the hyporheic proportion of TIA peaking in September at sites 1, 3 and 4 and in August at site 2. In contrast, at site 5, the hyporheic proportion was particularly high in July, remained high in August then declined sharply in September ($F_{3, 9} = 6.243$, $p = 0.014$; Figure 5.21).

Gammarus pulex

The hyporheic proportion of the *G. pulex* population was lowest at site 1 (0.08 ± 0.03) and highest at site 5 (0.30 ± 0.06; $F_{4, 15} = 4.719$, $p = 0.011$). Temporally, the hyporheic proportion was relatively low in May and June, intermediate in July and September and peaked in August; temporal change was not significant when all sites were considered ($F_{3, 57} = 2.397$, $p = 0.078$; Figure 5.21(ii); Table 5.20). However, the hyporheic abundance of *G. pulex* was temporally stable at site 1, and exclusion of this site resulted in the hyporheic proportion increasing significantly from 0.10 ± 0.03 in May and June to 0.27 ± 0.07 in August ($F_{3, 45} = 3.168$, $p = 0.033$; Table 5.20). The interaction between the hyporheic proportion and site was not significant.

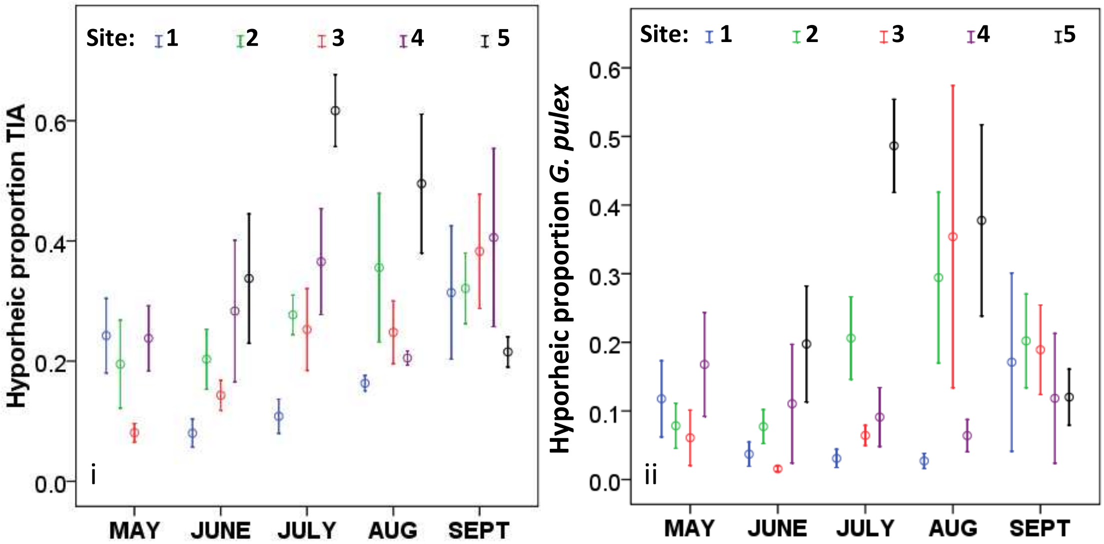


Figure 5.21: Mean ± 1 SE hyporheic proportion of the total (benthic + hyporheic) invertebrate community: i) total invertebrate abundance (TIA); ii) *Gammarus pulex*

Table 5.20: The hyporheic proportion of the total (benthic + hyporheic) invertebrate community present on the River Lathkill, May to September 2008.

	May	June	July	August	Sept	Temporal change
TIA	0.19 ± 0.03	0.21 ± 0.04	0.32 ± 0.05	0.29 ± 0.04	0.33 ± 0.04	*
Gammarus pulex	0.11 ± 0.03	0.09 ± 0.03	0.18 ± 0.04	0.22 ± 0.06	0.16 ± 0.03	* (site 1 excluded)
Polycelis felina	0.51 ± 0.11	0.41 ± 0.10	0.40 ± 0.09	0.57 ± 0.09	0.37 ± 0.09	ns
Oligochaeta	0.35 ± 0.08	0.26 ± 0.07	0.29 ± 0.07	0.27 ± 0.07	0.30 ± 0.05	ns

Values presented as mean ± 1 SE of all samples, n = 16 in May and n = 20 from June onwards. Temporal change analysed using one-way RM ANOVA, * indicates p <0.05, ns indicates p >0.05. See text for explanation of exclusion of site 1.

Polycelis felina

The mean hyporheic proportion of the total *P. felina* population varied between 0.35 ± 0.10 at site 3 to 0.57 ± 0.12 at site 5. Proportions were highly variable due to the population being patchily distributed and there were no significant spatial differences. Temporally, the hyporheic proportion was lowest in August and highest in September, but temporal change was not significant (Table 5.20).

Oligochaeta

The hyporheic proportion of the oligochaete population was considerably higher at site 3 (0.63 ± 0.07) compared with any other site (0.12-0.35 ± 0.03-0.06; $F_{4,\,15}$ = 8.334, p = 0.001). Overall, the hyporheic proportion varied between 0.26 ± 0.07 in

June and 0.35 ± 0.08 in May; temporal change was not significant overall or at any individual site (Table 5.20).

5.8 The invertebrate community at site 5 in 2009

Invertebrate samples collected during the second year of sampling at site 5 are investigated, to allow comparison with patterns observed in 2008.

5.8.1 Benthic invertebrate community

TIA peaked in June (633 ± 224 $0.1m^{-2}$) then declined moderately until September ($F_{1.611, 4.834} = 1.365$, $p = 0.327$). Mean taxon richness was highest in May and June (20.3 ± 0.8-0.9 taxa $0.1m^{-2}$) and lowest in July (15 ± 0.9 taxa $0.1m^{-2}$; $F_{2.192, 6.577} = 6.699$, $p = 0.025$). Neither the Simpson's diversity index nor the Berger-Parker dominance index experienced significant temporal change ($p \geq 0.303$). *G. pulex* dominated the benthic community, accounting for 56.4 % TIA and occurring at mean densities of 248 ± 24 $0.1m^{-2}$. Mean *G. pulex* abundance remained stable between May and September ($F_{1.502, 4.506} = 1.363$, $p = 0.328$). Of the other common taxa, only insects experienced significant temporal change in abundance.

5.8.2 Hyporheic invertebrate community

Temporal change in hyporheic TIA was not significant ($F_{4, 44} = 0.878$, $p = 0.485$). Hyporheic taxon richness, diversity and dominance were also comparable in all months. Community composition was similar to that recorded in 2008 and no non-insect taxa experienced significant temporal change in abundance.

5.8.3 Benthic invertebrate use of the hyporheic zone

G. pulex, *P. felina* and the Oligochaeta were the only non-insect taxa of occasional hyporheos to account for >1 % TIA; analysis of temporal change in the hyporheic proportion of total (benthic + hyporheic) populations was therefore restricted to these taxa. Neither the hyporheic proportion of TIA nor of any individual taxon varied significantly over time.

5.9 Invertebrate-environment relationships

For the multi-site 2008 data, canonical correspondence analysis (CCA) was used to investigate relationships between invertebrate communities and environmental variables in the benthic and hyporheic zones (aim 2, objective 4). Pearson correlation coefficients were also calculated to examine relationships between environmental variables and abundance of common taxa in both environments, but this analysis identified few relevant correlations and is not presented.

5.9.1 Canonical correspondence analysis: benthic community

Sufficient data was available for the inclusion of eight environmental variables, but DO concentration and % saturation were autocorrelated and the latter was excluded. A forward selection procedure using 999 iterations of the Monte Carlo random permutation test was applied to a preliminary CCA including three hydrological variables (water depth, mean flow velocity and wetted width) and four water chemistry variables (temperature, conductivity, pH and DO concentration). This analysis indicated that all contributed to the explanatory power of the model ($p \leq 0.0170$), and low variance inflation factors (≤ 2.0955) suggested that no variables were redundant with others in the dataset; all seven were therefore retained. Detrending was used to reduce an arch effect.

Spatial variability

Monte Carlo tests on the first canonical axis and all axes (the trace) were highly significant ($p = 0.001$), although the F ratio was higher for the first axis ($F = 9.408$) than the trace ($F = 4.212$). Eigenvalues indicated that all environmental gradients were relatively weak (Table 5.21). Axis 1 explained 9.7 % of the variance in the species data, was most strongly correlated with temperature and also had significant correlations with flow velocity, conductivity, wetted width and pH ($p < 0.01$; Figure 5.22). Axis 1 was primarily associated with longitudinal variability in water chemistry

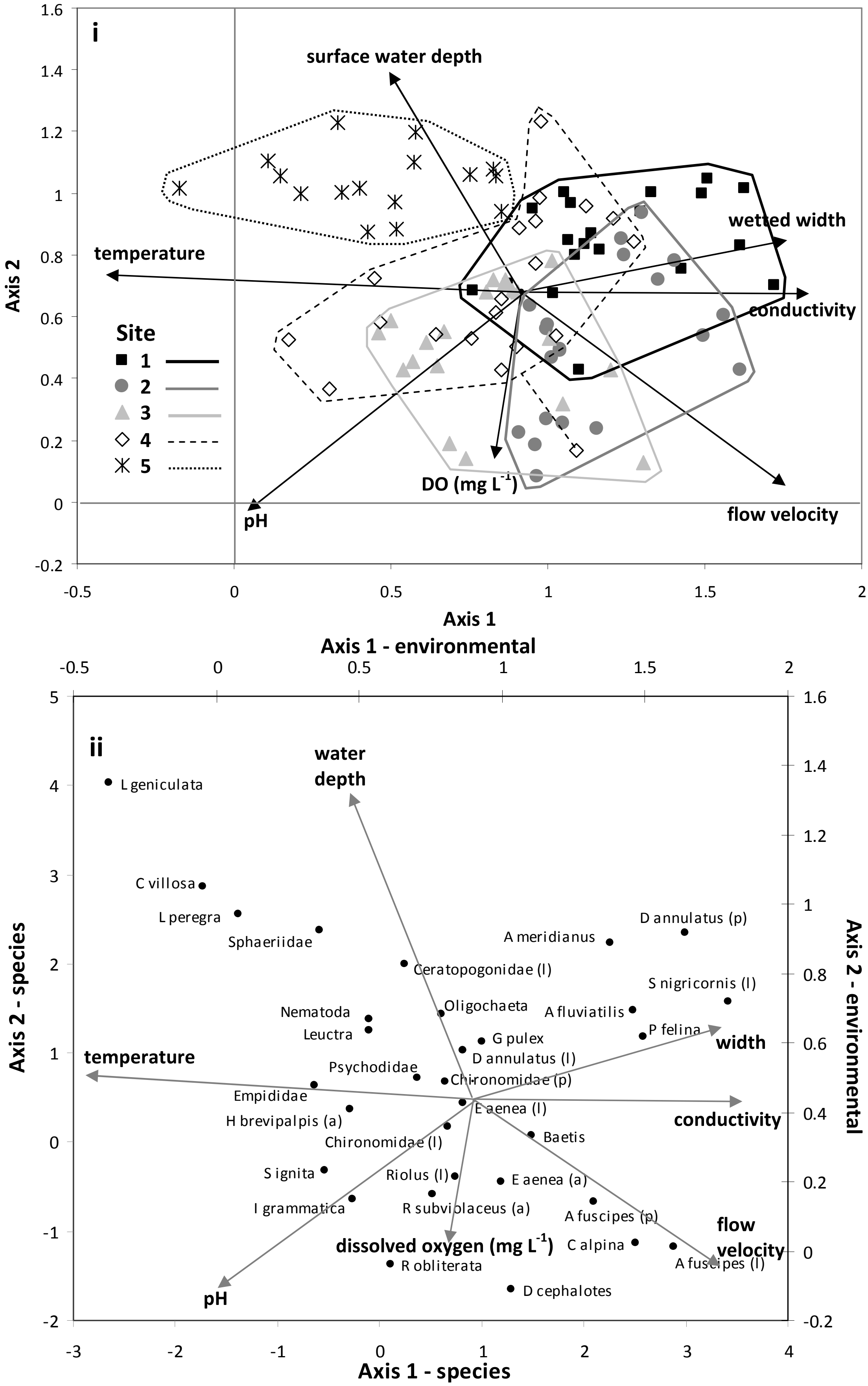


Figure 5.22: Detrended canonical correspondence analysis of benthic invertebrate and surface water data. Variability between sites: i) sample-environment biplot; ii) species-environment biplot.

related to groundwater influence, with a highly significant decrease in axis score from groundwater dominated site 1 (low temperature, high conductivity) to site 5 (high temperature, low conductivity; $F_{4,\,91}$ = 25.212, p <0.001; Figure 5.22(i)). Species with a preference for cool water, such as *Polycelis felina* and *Crenobia alpina*, scored highly on axis 1 (Figure 5.22(ii)). The second axis explained an additional 4.9 % of the variation, was most strongly correlated with depth, and was also correlated with velocity, conductivity and pH (p <0.01). This axis was therefore primarily associated with hydrological variability, with shallow, fast-flowing sites 2 and 3 having significantly lower axis 2 scores compared with deep, slow flowing site 5 ($F_{4,\,91}$ = 22.800, p <0.001; Figure 5.22(i)). Species such as *Leuctra geniculata* (Plecoptera) and *Chaetopteryx villosa* (Trichoptera) occurred primarily at site 5 and scored highly on axis 2 (Figure 5.22(ii)).

Table 5.21: Summary of detrended canonical correspondence analysis (DCCA) of benthic invertebrate community and environmental data from the River Lathkill

	Eigenvalues				**Cumulative % variance of species data**				**Cumulative % variance of species-environ. relation**			
Axis	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
ALL SITES	**0.135**	**0.068**	**0.038**	**0.017**	**9.7**	**14.6**	**17.3**	**18.4**	**38.2**	**60.9**	**0**	**0**
ALL SITES (covariable)	**0.074**	**0.044**	0.016	0.008	**7.4**	**11.8**	13.4	14.2	50.8	81.3	0	0

Temporal variability

DCCA was repeated with site as a covariable to allow observation of general patterns of temporal change (Figure 5.23). Monte Carlo tests indicated that explanatory variables associated with both the first canonical axis and the trace were highly significant (p = 0.001), with the F value being higher for the first axis (F = 6.743) than the trace (F = 2.703). However, eigenvalues indicated that all environmental gradients were weak (Table 5.21).

Axis 1 explained 7.4 % of the variance in the species data, was most strongly correlated with flow velocity and also had highly significant correlations with all other environmental variables except pH. Temporal change was significant on axis 1 ($F_{4,\,91}$ = 134.112, p <0.001), with axis scores declining gradually from May to July, primarily reflecting a decline in flow velocities. Axis 1 scores then peaked in

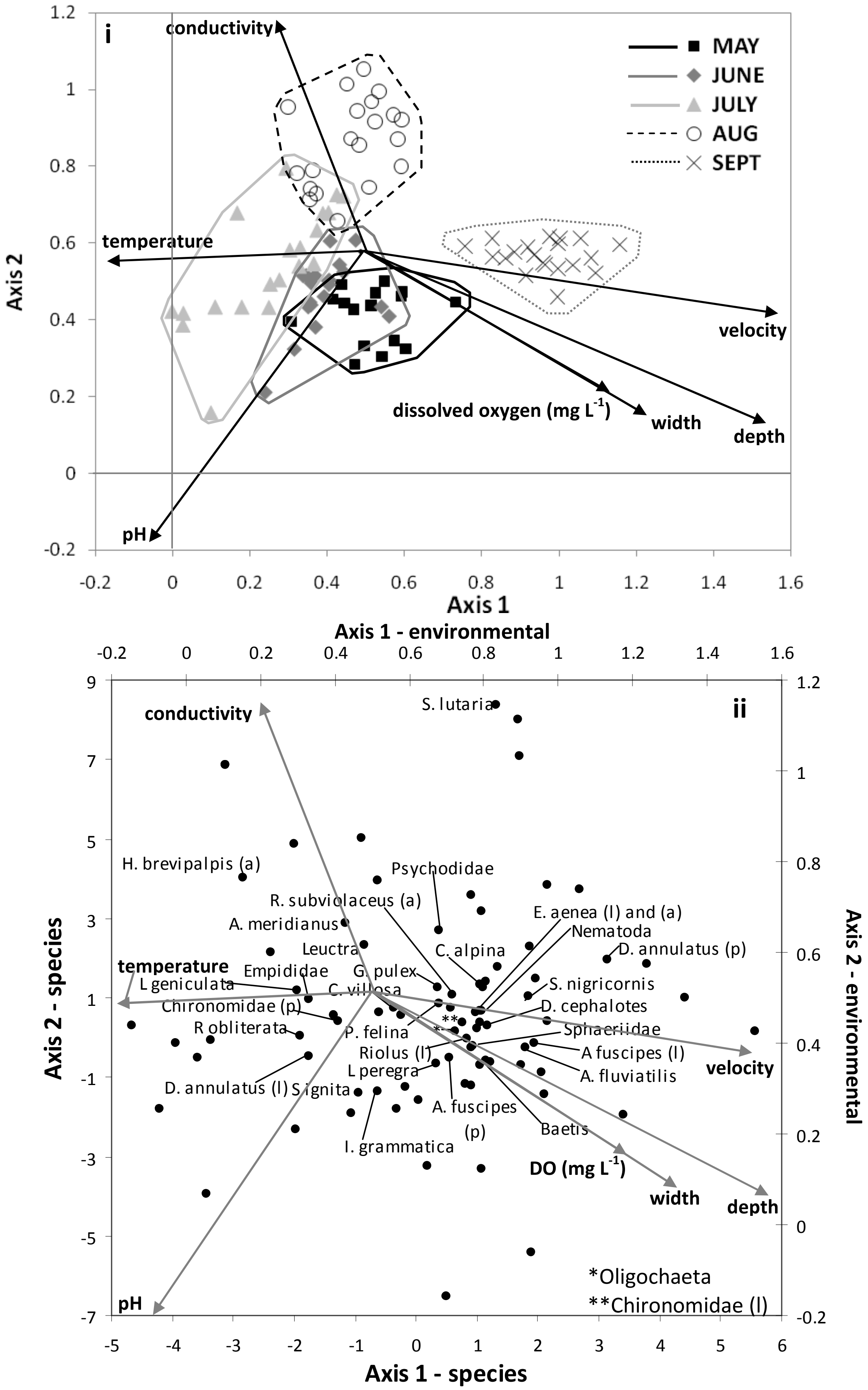


Figure 5.23: Detrended canonical correspondence analysis ordination of benthic invertebrate and surface water data, with site as a covariable. Temporal variability: i) sample-environment biplot; ii) species-environment biplot; several taxa occurring at low abundance are not labelled.

September when velocities were particularly high (Figure 5.23(i)). Most common taxa plotted near the centre of the species-environment ordination although some environmental preferences were apparent, for example *Helophorus brevipalpis* adults were most common in drying margins and positioning of this taxon indicates a preference for warm, shallow water (Figure 5.23(ii)). In contrast, taxa with preferences for cooler water (e.g. *Crenobia alpina*) and faster velocities (e.g. *Agapetus fuscipes* larvae) had higher axis 1 scores.

Axis 2 explained an additional 4.4 % of the variance and was most strongly correlated with pH and also conductivity; all other variables were more closely related to axis 1. Axis scores increased between May and July and were particularly high in August when pH was lowest and conductivity peaked ($F_{4, 91} = 56.077$, $p < 0.001$; Figure 5.23(i)). Most taxa had moderate axis 2 scores, reflecting the water chemistry recorded, whilst taxa with extreme scores (e.g. *Sialis lutaria*) were restricted to few samples (Figure 5.23(ii)).

Spatiotemporal variability

Whilst the original DCCA had distinguished between shallow, fast-flowing sites and deeper, slower sites, inclusion of site as a covariable indicated the community response to concurrent changes in all hydrological parameters as discharge (and therefore water chemistry) varied (Figure 5.23(ii)). Taxa such as *H. brevipalpis*, which benefitted from the decline in flow, could be distinguished from insects experiencing seasonal peaks in abundance in September despite the spate (*A. fuscipes* larvae, *D. annulatus* pupae; Figure 5.23(ii)).

5.9.2 Canonical correspondence analysis: hyporheic community

Data was available for five environmental variables: temperature, pH, conductivity, DO concentration and DO % saturation; this latter variable was excluded due to autocorrelation with DO concentration. A forward selection procedure using 999 iterations of the Monte Carlo random permutation test was applied to a preliminary CCA including the four variables; all were found to improve the fit of the model and all were retained. Detrending was used to eliminate an arch effect.

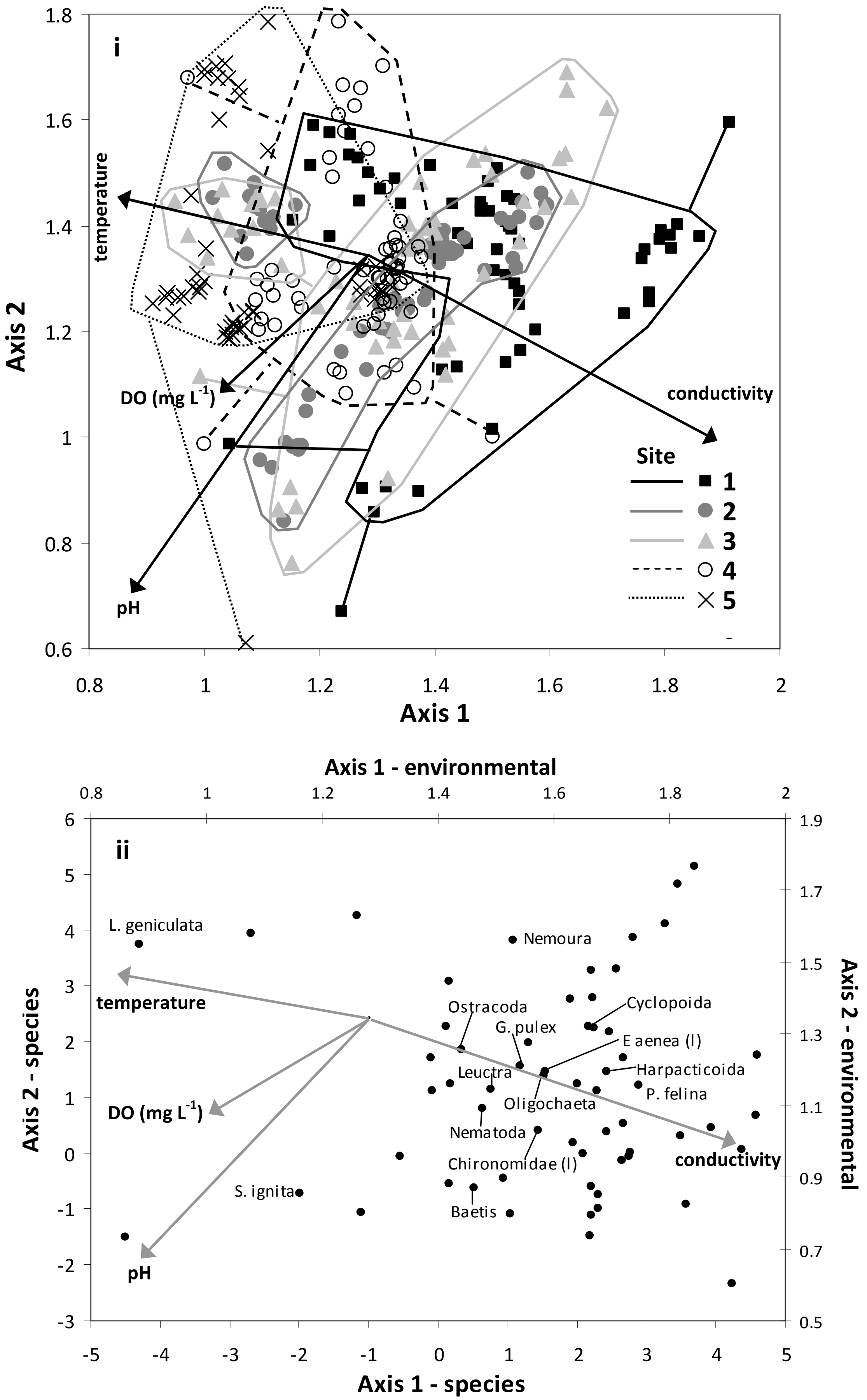


Figure 5.24: Detrended canonical correspondence analysis of hyporheic community and environment data. Spatial variability: i) sample-environment biplot; ii) species-environment biplot.

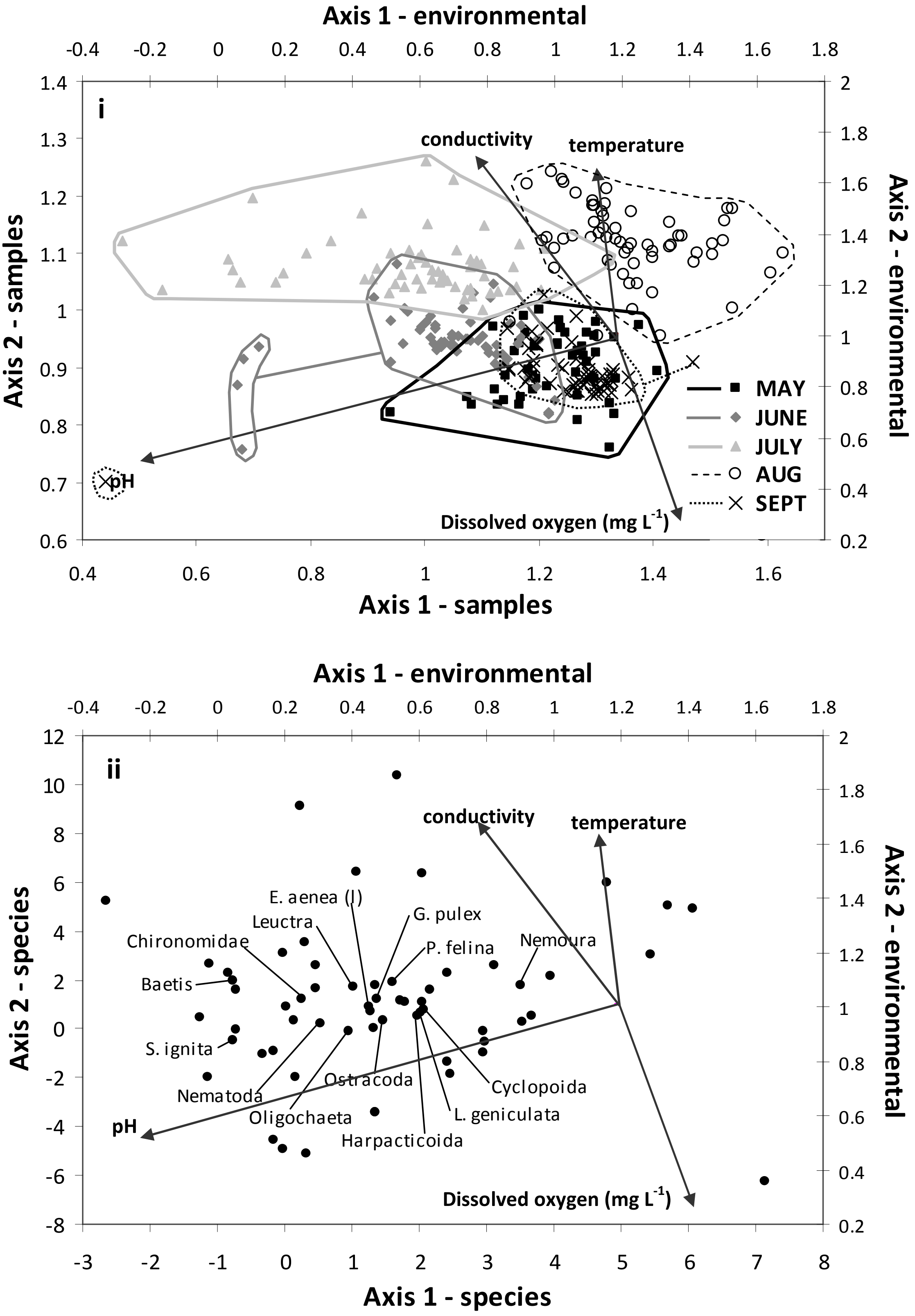


Figure 5.25: Detrended canonical correspondence analysis ordination of hyporheic invertebrate and water data, with site as a covariable. Temporal variability: i) sample-environment biplot; ii) species-environment biplot. Labelled species each comprised >0.5 % of the hyporheic fauna.

Spatial variability

Monte Carlo tests indicated the significance of both the first canonical axis (*F* ratio = 8.362, $p = 0.001$) and the trace (*F* ratio = 4.871, $p = 0.001$). Eigenvalues were particularly low on all axes, indicating weak environmental gradients (Table 5.22).

Axis 1 explained 2.9 % of the variance and was significantly correlated with all variables, particularly conductivity and temperature. As in surface water, axis 1 was primarily associated with longitudinal changes in groundwater dominance and water chemistry. Axis 1 scores were high at site 1, reflecting low temperatures and high conductivity and particularly low at site 5, where temperatures were high and conductivity low ($F_{4, 281} = 39.930$, $p < 0.001$; Figure 5.24(i)). Species characteristic of site 1 (e.g. *P. felina*) scored highly on axis 1 whilst those restricted to site 5 (e.g. *Leuctra geniculata*) had negative scores (Figure 5.24(ii)). Axis 2 explained 1.9 % of the species variation and had highly significant correlations with pH and, to a lesser extent, conductivity; spatial variability was not significant ($F_{4, 281} = 0.774$, $p = 0.543$). Most species had intermediate axis 2 scores, with those at the axis extremes represented by very few specimens (Figure 5.24(ii)).

Temporal variability

DCCA was repeated with site as a covariable to allow observation of overall temporal variability (Figure 5.25). Monte Carlo tests indicated the significance of both the first canonical axis (*F*-ratio = 6.766, $p = 0.001$) and the trace (*F*-ratio = 3.287, $p = 0.001$). Eigenvalues indicated that environmental gradients were particularly weak on all axes (Table 5.22). Axis 1 explained 2.4 % of the variance in the species data and had significant negative correlations with both conductivity and pH. Axis scores changed significantly over time ($F_{4, 281} = 88.631$, $p < 0.001$), declining gradually between May and July as pH and conductivity increased, peaking in August in response to a decline in pH then returning to May levels in September; the sample-environment biplot demonstrated complete overlap between samples from these two months(Figure 5.25(i)). Axis 2 explained a further 1.1 % of the species data variation, and was significantly correlated with all four measured variables, in particular conductivity. Axis 2 scores also changed significantly over time ($F_{4, 281} = 218.338$, $p < 0.001$ – note the higher *F* ratio in comparison with axis 1), increasing each month between May and August as conductivity increased and DO availability declined, before returning to May levels in September (Figure 5.25(i)).

Table 5.22: Summary of detrended canonical correspondence analysis of hyporheic invertebrate community and environmental data from the River Lathkill

	Eigenvalues				**Cumulative % variance of species data**				**Cumulative % variance of species data**			
Axis	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
ALL SITES	0.064	0.042	0.008	0.008	2.9	4.8	5.2	5.5	42.2	75.1	0	0
ALL SITES (covariable)	0.046	0.017	0.012	0.006	2.4	3.3	3.9	4.2	57.9	90.4	0	0

Common taxa all plotted towards the centre of the species biplot (Figure 5.25(ii)), indicating tolerance of the full range of environmental conditions reported. The most widespread taxa (i.e. Chironomidae, *G. pulex*) plotted centrally despite significant temporal changes in abundance. However, seasonal changes in the abundance of some insects were apparent on axis 1. *Baetis* species, for example, were most abundant in July and had a negative axis 1 score, whilst an August peak in *Nemoura* abundance was reflected by a relatively high score. In contrast, the axis 2 scores of all common taxa (>0.5 % TIA) fell within a small range (Figure 5.25(ii)).

Spatiotemporal variability

Comparison of the two ordinations (Figure 5.24; Figure 5.25) indicated that the principal control on community composition was variability in water chemistry related to groundwater influence. This was reflected in both longitudinal variability as groundwater dominance declined with progression downstream, and also in temporal variability as groundwater inputs became more influential at lower flows.

5.10 Spatial variability in invertebrate habitat

To improve understanding of spatial variability in refugium use by benthic invertebrates, two ‘stable’ habitat factors known to influence the composition of the hyporheic invertebrate community are examined: sediment grain size distribution and hydrologic exchange (aim 2, objective 5). Both parameters do vary over time, but are relatively constant compared to the hydrological and water quality variables measured each month.

5.10.1 Sediment composition

The grain size distribution of McNeil sediment samples collected from multiple sampling areas at each site was expressed as grain size classes ranging from <63 µm to >8 mm (Table 5.23). The finest size fractions (<63 µm, 63-125 µm) were comparable at all sites whilst significant spatial differences were observed in most other classes. These differences were related to the dominance of the 4-8 mm fraction at site 4 ($F_{4,11} = 8.618$, $p = 0.002$), the high proportion of grains >8 mm at sites 1-3 compared with sites 4 and 5 ($F_{4,11} = 12.862$, $p < 0.001$), and the corresponding high proportion of finer sediments (125 µm to 2 mm) at site 4 and 5 (Table 5.23). On site observations indicated homogeneity of sediment composition within the vertical profile at all sites.

Pearson correlation coefficients were calculated to assess the effects of sediment composition on hyporheic community metrics (Table 5.24). Invertebrate abundance was positively correlated with all size classes of 2-4 mm and below, this being significant for the 125-250 µm ($p = 0.001$) and 250-500 µm ($p = 0.015$) fractions. Additional analyses indicated that these correlations increased in strength with increasing hyporheic depth (data not shown). In contrast, correlations with mean taxon richness were negative (but weak and non-significant) at both extremes of the size class range (<63 µm, 63-125 µm and >8 mm) whilst relationships were positive for intermediate classes, with significance peaking at 1-2 mm ($p = 0.020$). Community dominance values were positively correlated with the finest size classes, this being significant for 125-250 µm and below ($p \leq 0.010$), whilst relationships with coarser fractions were not apparent. Investigation of relationships with the cumulative percentage of sediment below successive thresholds slightly strengthened the described correlations between sediment classes and dominance/abundance, whilst relationships with taxon richness became less distinct (Table 5.24).

Table 5.23: Grain size distribution of sediment samples from River Lathkill sites 1-5

	% sediment in each size class															
	Site 1				**Site 2**			**Site 3**		**Site 4**				**Site 5**		
Sampling area → Size class ↓	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**2**	**3**	**1**	**2**	**3**	**4**	**2**	**3**	**4**
<63 µm	1.3	0.8	0.6	1.1	0.9	0.4	0.5	0.8	0.7	1.5	0.9	0.9	0.8	1.5	0.9	1.0
63-125 µm	2.9	1.6	0.9	2.8	2.6	0.6	0.5	1.4	0.7	2.4	1.3	1.5	1.1	2.6	2.3	1.9
125-250 µm	2.9	1.8	0.9	2.8	4.0	1.2	1.3	2.0	1.0	5.6	2.8	3.5	2.2	6.4	5.4	4.1
250-500 µm	2.5	2.2	1.8	3.9	7.3	2.5	3.7	4.1	2.1	**11.0**	7.7	9.2	5.7	**10.3**	8.7	6.6
0.5-1 mm	2.1	3.4	4.8	6.5	8.9	4.1	8.8	9.4	5.4	**10.0**	**10.8**	**12.1**	9.5	**11.7**	**10.0**	**11.6**
1-2 mm	2.3	6.3	9.4	9.8	7.3	6.1	9.8	**12.4**	9.0	8.9	**11.5**	**11.7**	**11.7**	**12.8**	**11.3**	**16.2**
2-4 mm	3.6	**12.0**	**12.9**	**10.6**	7.8	**10.7**	**12.7**	**11.0**	**11.1**	**12.3**	**15**	**15.2**	**14.3**	**14.0**	**14.8**	**17.7**
4-8 mm	8.9	**18.8**	**17.4**	**11.8**	**13.1**	**17.7**	**15.1**	**11.2**	**13.2**	**21.8**	**25.1**	**23.8**	**21.2**	**11.0**	**13.5**	**8.9**
>8 mm	**74.8**	**53.2**	**51.3**	**50.8**	**47.9**	**56.8**	**47.7**	**47.7**	**56.8**	**26.5**	**24.9**	**22.1**	**33.5**	**29.6**	**33.0**	**24.0**

Size classes comprising >10 % of the sediment weight in bold; dominant size class is highlighted.

Table 5.24: Pearson correlation coefficients between sediment composition and hyporheic invertebrate community metrics

Size class	**TIA[1]**	**Taxa[2]**	**Dominance[3]**	**Cumulative size class**	**TIA[1]**	**Taxa[2]**	**Dominance[3]**
<63 µm	0.208	-0.082	0.385**	**<63 µm**	0.208	-0.082	0.385**
63-125 µm	0.155	-0.165	0.322**	**<125 µm**	0.175	-0.146	0.351**
125-250 µm	0.356**	0.058	0.228*	**<250 µm**	0.300**	-0.025	0.293**
250-500 µm	0.275**	0.118	-0.126	**<500 µm**	0.305**	0.056	0.215
0.5-1 mm	0.190	0.209	-0.046	**<1 mm**	0.283**	0.122	0.127
1-2 mm	0.186	0.266*	-0.091	**<2 mm**	0.282*	0.180	0.072
2-4 mm	0.214	0.249*	-0.103	**<4 mm**	0.286*	0.212	0.031
4-8 mm	-0.049	0.052	-0.024	**<8 mm**	0.228*	0.201	0.018
>8 mm	-0.223	-0.200	-0.002	**-**	-	-	-

[1]Total invertebrate abundance; [2]Mean taxon richness; [3]Mean Berger-Parker dominance index. All hyporheic depths combined, ****p* **≤0.05; ** *p* **≤0.01**

5.10.2 Hydrologic exchange

Mini-piezometers installed at sites 2 and 5 suggested that the dominant direction of hydrologic exchange at both sites was strongly downwelling. However, such a result may also be obtained if sediments with low hydraulic conductivity occur between the piezometer base and the sediment surface (Boulton, 2007b) and these results should therefore be treated with caution. Other evidence has therefore been collated to provide an indication of the direction of hydrologic exchange at each site. Site 1 was demonstrably strongly upwelling; groundwater springs are located adjacent to the site and upwelling water was directly observed during sample collection. Additional evidence of upwelling water is seen in the water chemistry, with high conductivity, low water temperatures and low DO concentrations characterising site 1. Mini-piezometer data indicated that site 2 was strongly downwelling, and water chemistry lacked the groundwater signature observed at site 1. This site is located at the downstream end of the perennial reach and flow may be maintained despite transmission losses due to inputs from upstream. However, observation of an obligate groundwater taxon (*Antrobathynella stammeri*) from a 20 cm hyporheic sample suggests some influence of upwelling water. Historic intermittency at sites 3-5 suggest that downwelling transmission losses to underlying drainage levels are not typically offset by direct inputs of upwelling groundwater at these sites, with most water instead originating from sources upstream. An intermittent spring is located between sites 3 and 4, but particularly low conductivity and moderate water temperature at site 4 indicate that this was of little influence. In addition to mini-piezometer data, evidence that site 5 is strongly downwelling is provided by water temperature data was as warm in the hyporheic zone (11.95 ^{o}C) as in surface water (11.98 ^{o}C).

5.11 Discussion

In this section, the key results obtained from the River Lathkill sampling campaign are discussed with reference to the aims of the thesis (section 1.2). First, the environmental conditions experienced are considered as potential environmental stressors; second, the response of benthic community to the identified stressors is examined; and third, benthic invertebrate use of the hyporheic refugium is investigated in relation to both habitat- and disturbance-related parameters.

5.11.1 Identification of potential environmental stressors

The first aim of this chapter (section 5.2) was to examine changes in surface hydrology, related changes in environmental variables, and consequent changes in biotic factors with the potential to increase stress for benthic invertebrates.

Variation in surface hydrology in long-term context

Hydrological conditions on the Lathkill comprised a four-month flow recession (including habitat contraction), localised streambed drying, and a high-magnitude spate (Figure 5.2). Whilst each of these hydrological elements had the potential to detrimentally affect the benthic invertebrate community, conditions were moderate compared with a ‘normal’ year. At the low flow end of the hydrological continuum, flow duration analysis and local observations (P. Bowler, pers. comm.) indicate that flow recession typically precedes complete streambed drying at sites 3-5, whilst in the study year surface water remained connected along the length of the river (Figure 5.3). At the other extreme, the spate event was high-magnitude, and the rate of change was very rapid. Such high-flow events are common on the Lathkill, with an event of similar magnitude occurring in the winter preceding the study (data not shown), and therefore the invertebrate community may include flood-resistant taxa (Lytle and Poff, 2004); nonetheless, a spate of the magnitude recorded is a particularly stressful event for instream fauna (Death, 2008).

Effects of flow variability on instream habitats

During the flow recession, pronounced reductions in width and depth lowered the water volume and increased the influence of external factors (e.g. insolation and groundwater; Webb, 1996; Caruso, 2002; Dewson *et al*., 2007a). Concurrent declines in oxygen availability were particularly pronounced at groundwater-fed site 1 (Figure 5.6); however, significant changes in oxygen and other water chemistry parameters were only minor and unlikely to have biotic effects (Winter *et al*., 2002; Table 5.7). Declines in depth and width during the flow recession also exposed mid-channel and marginal benthic sediments (Table 5.6; Figure 5.5), which can result in stranding of invertebrates in exposed areas (Extence, 1981; Lancaster, 2008) and can concentrate mobile taxa into a smaller submerged area (Fritz and Dodds, 2004; Dewson *et al*., 2007b). Only two marginal sampling points were located in areas that dried and it is likely that pronounced shifts in community composition would be restricted to these areas (Boulton, 2003). Flow velocities also declined during the flow recession (Figure 5.4(ii)), but these only resulted in localised ponding at site 4, whilst site 5 was slow-flowing throughout the study, and other sites retained moderate to fast-flowing habitat. Therefore, during the flow recession, habitat availability was widely reduced whilst declines in habitat heterogeneity were limited (cf. McIntosh *et al*., 2002; Dewson *et al*., 2007a).

In contrast to the flow recession, the spate maximised habitat availability (Table 5.6), increased flow velocities (Figure 5.4), and reduced groundwater dominance (Boulton and Hancock, 2005; Figure 5.10). However, high velocities were recorded several days after spate flows peaked, and it is probable in a constrained reach that higher discharge was accompanied by faster velocities in the preceding days (Lancaster, 1999). The supposition is supported by evidence of bedload movement in several sampling areas, including both scouring and deposition of fine material. High velocities, high shear stress and mobile sediment typically combine to displace or damage benthic invertebrates during spates (Strommer and Smock, 1989; Palmer *et al*., 1992; Matthaei *et al*., 1999; Maier, 2001), and the CCA of temporal variability identified high September velocities as particularly influential in determining benthic community composition (Figure 5.23).

Potential effects of flow variability on biotic interactions

During the spate, the harsh environmental conditions are likely to have been the most influential determinants of benthic community composition (Menge and Sutherland, 1976; Peckarsky, 1983; Townsend *et al*., 2003), whilst biotic factors are likely to have increased in importance between May and August (Lancaster, 1996; Death, 2010); the following discussion will therefore focus on this period of flow recession.

Total abundance of benthic invertebrates did not experience significant temporal change as flow declined (Figure 5.14(i)), but this apparent stability masked a significant threefold increase in the abundance and dominance of the amphipod *Gammarus pulex* between May and August, which reflected increases at all sites (Figure 5.15(i)). *G. pulex* is a highly mobile taxon (Hughes, 1970; Pearson and Jones, 1987; Elser, 2001) and changes in its abundance closely reflected changes in habitat availability; increased population densities are therefore likely to reflect concentration into a declining habitat area rather than a numerical increase in abundance (Covich *et al*., 2003; Fritz and Dodds, 2004). This suggestion is also supported by the stable *G. pulex* population densities recorded at site 5 in 2009 (section 5.8.1), when no reduction in habitat availability was recorded (section 5.45). *G. pulex* is a highly competitive taxon which exerts an influence on the benthic community through competition for space and resources Dick *et al*., 1990; Graça *et al*., 1993) as well as through predation (including intraspecific predation (cannibalism); Dick, 1995; MacNeil *et al*., 1997; Kelly *et al*, 2002), and an increase in its abundance is therefore likely to intensify density-dependent biotic interactions (Elton, 1949; Savage, 1996). This is particularly the case considering the high population densities *G. pulex* recorded (mean densities of 2321 m^{-2} and peak densities reaching 6480 m^{-2} recorded in August; Figure 5.15(i)), which are comparable with some studies (e.g. Mortensen, 1982) and considerably higher than many others in comparable habitats (Macan and Mackereth, 1957; Crane, 1994). In addition, the flatworm *Polycelis felina* occurred at high abundance when habitat availability was low at sites 1 and 2 between June and August (Figure 5.15(ii)). *P.*

felina is predator which consumes a range of taxa including gammarids (MacNeil *et al*., 1999). It is therefore suggested that whilst the flow recession represented a period of moderate hydrological conditions, the contraction in submerged habitat availability concentrated competitive benthic taxa into a smaller space, potentially causing a considerable increase in the strength of biotic interactions.

5.11.2 Benthic community response to hydrological variability

The second aim of this chapter (section 5.2) was to examine how the benthic invertebrate community responded to factors identified as potential stressors. In this section, changes in the community present in the benthic sediments are considered.

Temporal change in benthic community composition

The CCA investigating temporal relationships between community composition and environmental parameters showed that the community changed gradually between May and August and was distinct from all preceding months in September (Figure 5.25). Community change between May and August largely reflected the gradual increase in *G. pulex* population densities and community dominance, as previously discussed. Such increases in occurrence of dominant taxa have been proposed to reduce overall community diversity through exclusion of less competitive taxa (Hardin, 1960; Connell, 1978), although evidence of such exclusions are limited (Reice, 1981; Death and Winterbourn, 1995). Whilst Simpson's diversity fell between May and August (Figure 5.14(iv)), this only reflected a reduction in community evenness, with no accompanying decline in taxon richness (Figure 5.14(ii)). Indeed, following exposure of marginal benthic sediments in August localised increases in taxon richness were recorded, reflecting colonisation of this new habitat type by semi-aquatic Coleoptera; such positive relationships between habitat complexity/heterogeneity and taxonomic richness are well understood in stream ecosystems (Townsend, 1989; Vinson and Hawkins, 1998).

The separation of September samples on the CCA ordination reflected the depauperate community present in the aftermath of the spate (Figure 5.23). The low invertebrate abundance recorded in September is typical of post-spate communities (Figure 5.14(i); Scrimgeour and Winterbourn, 1989; Olsen and Townsend, 2005; Death, 2008), with factors including the rapid rate at which discharge increased and the mobilisation of surface sediments likely to increase detrimental impacts on the community (Matthaei *et al*., 1999). Whilst the impact of the Lathkill spate was considerable, many studies have noted more pronounced declines than those reported here (Fritz and Dodds, 2004; Olsen and Townsend, 2005). By displacing competitive taxa, spates can reset community trajectories to an earlier successional stage (Fisher *et al*., 1982; Junk *et al*., 1989; Lake, 2000), and in the Lathkill the spate increased community diversity since taxon richness fell only slightly and community evenness increased due to a disproportionately large decline in *G. pulex* (Figure 5.14(ii); Figure 5.14(iv); Figure 5.15(i)).

5.11.3 Benthic invertebrate use of the hyporheic zone

Three conditions were identified as stressors of benthic invertebrates: the proposed intensification of biotic interactions following habitat contraction, localised drying in two sampling areas, and the spate. The hyporheic zone had the potential to act as a refugium in all cases since invertebrate population densities were lower during flow recession, free water was retained following surface drying, and disturbing forces are likely to have been of relatively low strength during the spate. To address aim 2 (objectives 2 and 3; section 5.2), this section examines use of the hyporheic zone by benthic invertebrates during each adverse condition.

Use of the hyporheic refuge following habitat contraction

Previous studies have found no evidence that benthic invertebrates use the hyporheic zone refugium during low flows, possibly because conditions remain favourable in the benthic sediments (James *et al*., 2008; James and Suren, 2009). However, in the Lathkill, a month after *G. pulex* population densities started to rise in the benthic sediments, the taxon started to become significantly more abundant in the hyporheic zone (Figure 5.20(i)). Concurrently, the proportion of the total population inhabiting the hyporheic sediments increased (Figure 5.21(ii)). The combination of increases in hyporheic abundance and proportion suggested active migration into the hyporheic zone rather than passive range extension of an expanding population (Wood *et al*., 2010; Figure 7.4). *P. felina* also became particularly abundant in the hyporheic zone in August whilst benthic densities remained stable, although an increase in the hyporheic proportion of the population was moderate (Table 5.20). Whilst reports of predation by *P. felina* on *Gammarus* are common (Reynoldson, 1981; MacNeil *et al*., 1999), there is also some (uncertain) evidence of the shrimp predating the flatworm (Davies and Reynoldson, 1969), and *P. felina* may therefore have migrated into deeper sediments in response to peak *G. pulex* densities.

Use of the hyporheic refuge following marginal streambed drying

Streambed drying only affected one site 3 and one site 4 marginal sampling area in August. No consistent patterns of increased taxon abundance were observed in hyporheic samples from beneath these sampling areas, indicating that the hyporheic zone was not a drying refugium; adjacent, laterally and longitudinally connected areas of the surface channel may have been preferable alternatives. However, taxon richness was very high in the hyporheic zone below dry surface sediments, with additions to the typical hyporheic community including unusually high densities of adults and larval riffle beetles (Elmidae). This indicated possible taxon-specific vertical migrations, but further field observations/experimental work would be required to substantiate this suggestion.

Use of the hyporheic zone following the spate

Following the spate, declines in invertebrate abundance were almost as severe in the hyporheic zone as in the benthic sediments (Figure 5.19(i)). Therefore, not only did benthic invertebrates not migrate into the hyporheic zone as surface flow increased, but many existing hyporheic inhabitants were not protected. High flows are archetypal events during which the hyporheic zone is proposed to act as a refugium (Williams and Hynes 1974; Boulton *et al*., 2004), due to relative sediment stability and low flow velocities limiting invertebrate displacement (Jones and Holmes, 1996; Brunke and Gosner, 1997). However, many previous studies have also reported declines in hyporheic abundance following a spate, this being attributed to environmental factors such as low sediment stability or an upwelling direction of hydrologic exchange (Dole-Olivier *et al*., 1997), or to disturbance related parameters such as spate onset being too rapid for a behavioural response (Imbert and Perry, 1999; Gayraud *et al*., 2000). On the Lathkill, the hyporheic zone had been demonstrated as a refugium in the months preceding the spate event, suggesting that features of the interstitial environment were adequate to support benthic invertebrates. However, whilst the hyporheic sediments are stable compared to those at the surface, shallower sediments can nonetheless be altered by high flows (Matthaei *et al*., 1999), as evidenced by bedload movement on the Lathkill. The disturbing forces themselves may therefore reduce the capacity of the hyporheic zone to act as a refugium during an event, and in addition may influence the invertebrate capacity to respond. Despite this absence of active refugium use, the proportion of the total invertebrate community resident in the hyporheic zone peaked in September, highlighting the additional importance of the hyporheic zone as a passive refugium (see section 7.11).

5.11.4 Spatial variability in the hyporheic refugium

The final objective of this chapter (aim 2, objective 5; section 5.2) was to relate spatial variability in the use of the hyporheic refugium to environmental factors controlling its fitness for purpose, including historic flow regime, water quality, sediment composition and direction and strength of hydrologic exchange. During the spate, the hyporheic zone was not actively used as a refugium at any site, which, considering the notorious heterogeneity of the hyporheic zone (Lake, 2000; Malcolm *et al*., 2004; Lancaster, 2008), indicated the overriding importance of disturbance-related parameters in determining community response. In contrast, migrations into the hyporheic sediments did occur during the flow recession, but refugium use varied between sites; the following discussion of this spatial variability is therefore focused on this hydrological element, and refers to refugium use by *G. pulex*, which was ubiquitous at high abundance in the benthic sediments.

At site 1, despite considerable habitat contraction from June (Table 5.6) and an associated increase in the benthic abundance of *G. pulex* (Figure 5.15(i)) the hyporheic abundance of this taxon was consistently low in all months (Figure 5.20(i)). This is particularly surprising considering that *G. pulex*'s benthic abundance was significantly higher at site 1 than at any other site, reaching >5000 m^{-2} in all months (Table 5.14). Site 1 is perennial due to groundwater inputs from springs located directly adjacent to sampling areas, and strongly upwelling water could be observed in some hyporheic sampling wells. The influence of groundwater on hyporheic water chemistry was also apparent, with low oxygen content and high conductivity recorded (Table 5.8; Malcolm *et al.*, 2004; Marmonier *et al.*, 2010). The direction and strength of hydrologic exchange has previously been noted as a determinant of hyporheic community composition (Marmonier *et al.*, 2010) and refugium use, with upwelling water reducing benthic inhabitation of the hyporheic zone due to low oxygen, low organic matter availability and the direction of water movement (Dole-Olivier *et al.*, 1997). The proportion of fine sediment in bulk sediment samples was particularly low at this site (probably due to continual flushing by upwelling water; Table 5.23; Brunke and Gosner, 1997), which can promote refugium use due to high water quality and habitat availability (Findlay, 1995; Wu, 2000); surprisingly, however, monthly measurements of hyporheic fine sediment concentrations were relatively high at site 1 (Figure 5.8). Regardless, any benefits of upwelling water appear to have been outweighed by the direction and/or strength of water movement and/or low hyporheic oxygen content.

Increases in the hyporheic abundance and proportion of the *G. pulex* population were observed at all other sites (2-5; Table 5.20), and environmental conditions are essentially the opposite of those described at site 1. Sites 3-5 are strongly downwelling, as evidenced by mini-piezometer data as well as the typical flow permanence regime. Whilst site 2 maintains perennial surface flow due to groundwater inputs from upstream, the site is located at the downstream extremity of the perennial reach and mini-piezometer data indicates that downwelling water dominated the direction of hydrologic exchange (section 5.10.2). Downwelling water can increase the benthic proportion of the invertebrate assemblage resident with the hyporheic zone (Davy-Bowker *et al*., 2006) and can also promote refugium use), since water chemistry is similar to the surface stream, oxygen content is typically high, there are regular inputs of organic matter food resources, and the direction of hydrologic exchange facilitates downwards migration (Dole-Olivier *et al*., 1997). The proportion of fine sediment present at these sites was relatively high, exceeding 30 % at sites 4 and 5 (Table 5.23), but this does not appear to have offset the various advantages of downwelling water.

5.11 Summary

The spate, habitat contraction and marginal streambed drying were identified as adverse conditions with the potential to stress benthic invertebrates, and the community responded to all conditions: reductions in abundance followed the spate and during drying, whilst increased population densities (and inferred intensification of biotic interactions) followed habitat contraction. The hyporheic zone was a potential refugium during all identified stressors, but increases in the hyporheic abundance and proportion of benthic invertebrates were only recorded during habitat contraction. In contrast to previous work conducted during low flows (James *et al*., 2008; James and Suren, 2009; Stubbington *et al*., 2009a), this study linked a gradual decline in discharge to an increase in benthic invertebrates in the hyporheic zone, and it is suggested that subsurface sediments provided protection from intensified biotic interactions in the surface stream. However, the hyporheic zone was a spatially variable refugium, and in particular no increase in benthic abundance was observed at a strongly upwelling site; this is attributed to a combination of low oxygen availability, and the direction of water movement. Despite the expectation that the hyporheic zone refuge would be used during the spate, a substantial decline in invertebrate abundance was recorded in both benthic and hyporheic habitats. The hyporheic zone nonetheless acted as a passive refugium during the spate by increasing overall community survival and providing a potential source of recolonists to the surface sediments.

6. Invertebrate community response to flow variability: the River Glen

6.1 Introduction

This chapter considers the responses of invertebrates in the benthic and hyporheic sediments to variation in surface flow on the River Glen. Discharge in the Glen is groundwater-dominated, but variation in the underlying geology (see Chapter 3) makes the flow regime responsive to rainfall in some reaches. During the study period (May-September 2008), hydrological conditions included a series of low-magnitude high-flow events, a two-month low flow period, habitat contraction and short-duration streambed drying. In total, 312 invertebrate samples were collected over five months, comprising 80 each from the benthic sediments and from hyporheic depths of 10 cm and 20 cm, and 72 samples from a depth of 30 cm (this difference being due to difficulties installing sampling wells). Temporal changes in benthic and hyporheic community composition are examined in relation to surface flow variability and consequent changes in habitat availability and water quality. Particular consideration is given to the occurrence of benthic invertebrates in the hyporheic zone, and use of this habitat is related to both temporally variable environmental factors (e.g. hydrology and water quality) and relatively stable habitat parameters (hydrologic exchange and sediment composition).

6.2 Aims and objectives

This chapter examines changes in the composition and distribution of invertebrate communities in the benthic and hyporheic sediments of the River Glen during a period of variable surface flow. The specific aims and objectives of this chapter are as follows:

Aim 1: Identify hydrological conditions and related changes in both environmental and biotic variables with the potential to stress benthic invertebrates.

Objectives

1. Examine variation in surface hydrology, using discharge data and by measuring site-specific instream variables.
2. Set the hydrological conditions experienced in context using long-term data.
3. Determine changes in habitat availability resulting from variation in surface flow.
4. Investigate changes in water quality parameters related to surface flow variation.
5. Use multivariate analysis to determine the principal environmental gradients.
6. Analyse changes in the abundance of taxa with the potential to influence the strength of biotic interactions (such as predation and competition) in the benthic sediments.

Aim 2: Examine invertebrate community responses to identified potential stressors, including changes in the use of the hyporheic zone by benthic invertebrates.

Objectives

1. Investigate temporal change in invertebrate community composition in the benthic and hyporheic zones using community metrics and multivariate analysis.
2. Identify temporal changes in the abundance of common benthic taxa in the surface sediments and the hyporheic zone.
3. Analyse temporal changes in the proportion of the total (benthic + hyporheic) community resident in the hyporheic zone.
4. Examine relationships between environmental conditions and community metrics to infer drivers of community change.
5. Investigate spatial variability in the suitability and use of the hyporheic refugium, with reference to historic flow regime, stable habitat parameters and temporally variable environmental factors.

6.3 Meteorological conditions and hydrological response

To address the first aim (objective 2), the prevailing meteorological conditions during the study are presented. Air temperature and rainfall are described and compared with long-term averages (LTA), and streamflow response to precipitation is examined using rainfall and discharge data (objective 1).

6.3.1 Air temperature

Air temperature showed a sharp increase between mid-April and mid-May, a second more gradual increase until July/August, then a decline in September (Figure 6.1; Table 6.1; also see section 4.8.1). The 12-hourly minimum temperatures exceeded zero in mid-May, then remained >5 °C for the rest of the study (Figure 6.1); 12-hourly maxima exceeded 24 °C every month from May onwards, but such high temperatures were only common in late July (Figure 6.1). Monthly mean temperatures increased each month between April and July, peaking in August (Table 6.1).

Table 6.1: Air temperature in the Glen catchment, April to September 2008, in comparison with long-term average conditions

	Air temperature (°C)						
	Maxima			Minima			Mean*
	Daily absolute§	Daily mean§	LTA**, Daily mean	Daily absolute§	Daily mean§	LTA**, Daily mean	Monthly
April	21	12.9	11.7	-1.9	3.4	4.0	7.65
May	25.3	19.1	15.4	1	8.3	6.7	12.6
June	24.6	19.2	18.3	5.5	9.9	9.7	14.3
July	28.1	22.0	21.0	8	12.2	11.9	16.4
August	24.9	21.0	20.9	8.1	13.4	11.8	16.8
Sept	20.7	18.0	17.7	4.6	9.6	9.8	13.6

§12-hour minimum/maximum values from Monks Wood (TL200801, 40 km to the south)

* calculated using weekly MORECS data (NERC, 2009); Figure 6.1 and section 4.8.1 provide details.

** Long-term averages (LTA, 1971-2000) for Waddington, 40 km to the north (Met Office, 2009c)

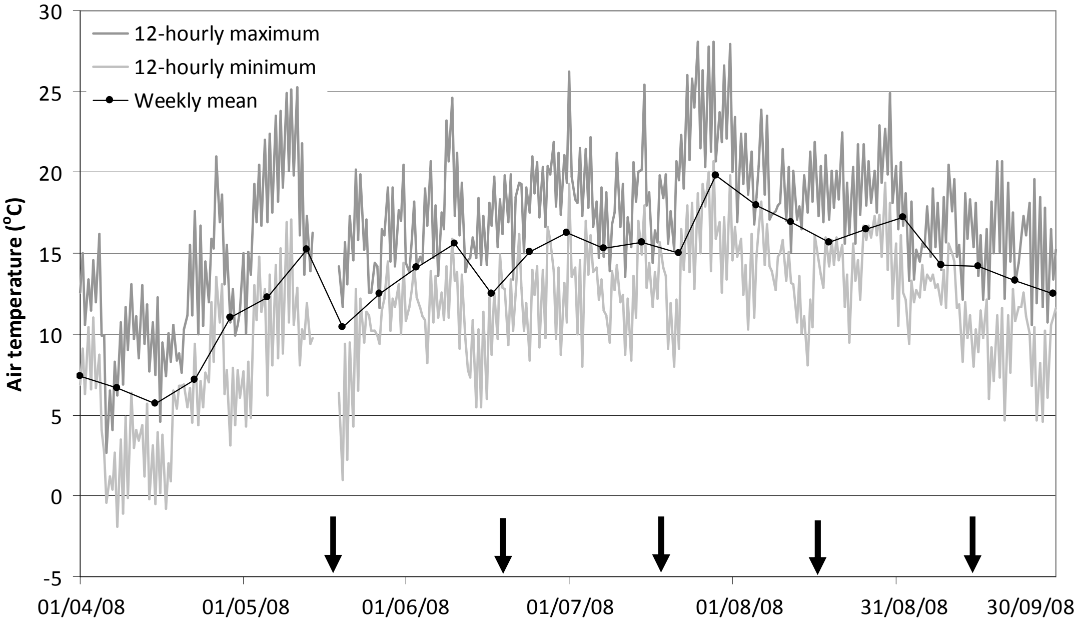


Figure 6.1: 12-hour minimum and maximum air temperature at Monks Wood and weekly mean air temperature for the Glen catchment region, April-September 2008. Arrows indicate sampling dates.

At a regional scale (the Midlands, East Anglia and Eastern England), 2008 daily mean minimum and maximum temperatures were ~2 °C above the LTA (1971-2000) in May, and close to the LTA between June and September (Met Office, 2009d). Comparison of the Monks Wood data with long-term (1971-2000) records from Waddington (SK988643, 40 km to the north of the Glen at a similar elevation; Met Office 2009c) indicated that mean daily maximum temperature in the Glen catchment exceeded the LTA throughout the study, by 3.7 °C in May and by ≤1 °C in later months (Table 6.1). Mean daily minimum temperatures were also above the LTA between May and August, and close to the LTA in September (Table 6.1).

6.3.2 Rainfall and streamflow response

Following recharge of the underlying aquifer by above-average rainfall between January and April 2008 (BADC, 2009), streamflow responded to minor precipitation inputs in May and June (Figure 6.2). This response was particularly pronounced in early June, when streamflow temporarily increased by between four- and ten-fold above baseflow on three successive occasions in the West Glen (Figure 6.2(i)), and by 18- to >100-fold on two occasions in the East Glen (Figure 6.2(ii)). These events were followed by rainfall below the 1980-2008 average in June and July (BADC, 2009) during which baseflow was stable on the West Glen at Little Bytham and declined (briefly to zero) at Manthorpe on the East Glen (Figure 6.2). Exposure of marginal areas was observed at site 4 in mid-July and complete streambed drying occurred in late July and again in early September at site 4. August rainfall was almost twice the 1980-2008 monthly average (104.3 mm compared with 58.9 mm; BADC, 2009), resulting in some small increases in streamflow. The cause of the streamflow fluctuations observed on the West Glen hydrograph (Figure 6.2(i)) during baseflow conditions are not known but may be due to abstractions associated with local quarrying activity (Ian Gray, Environment Agency, pers. comm.).

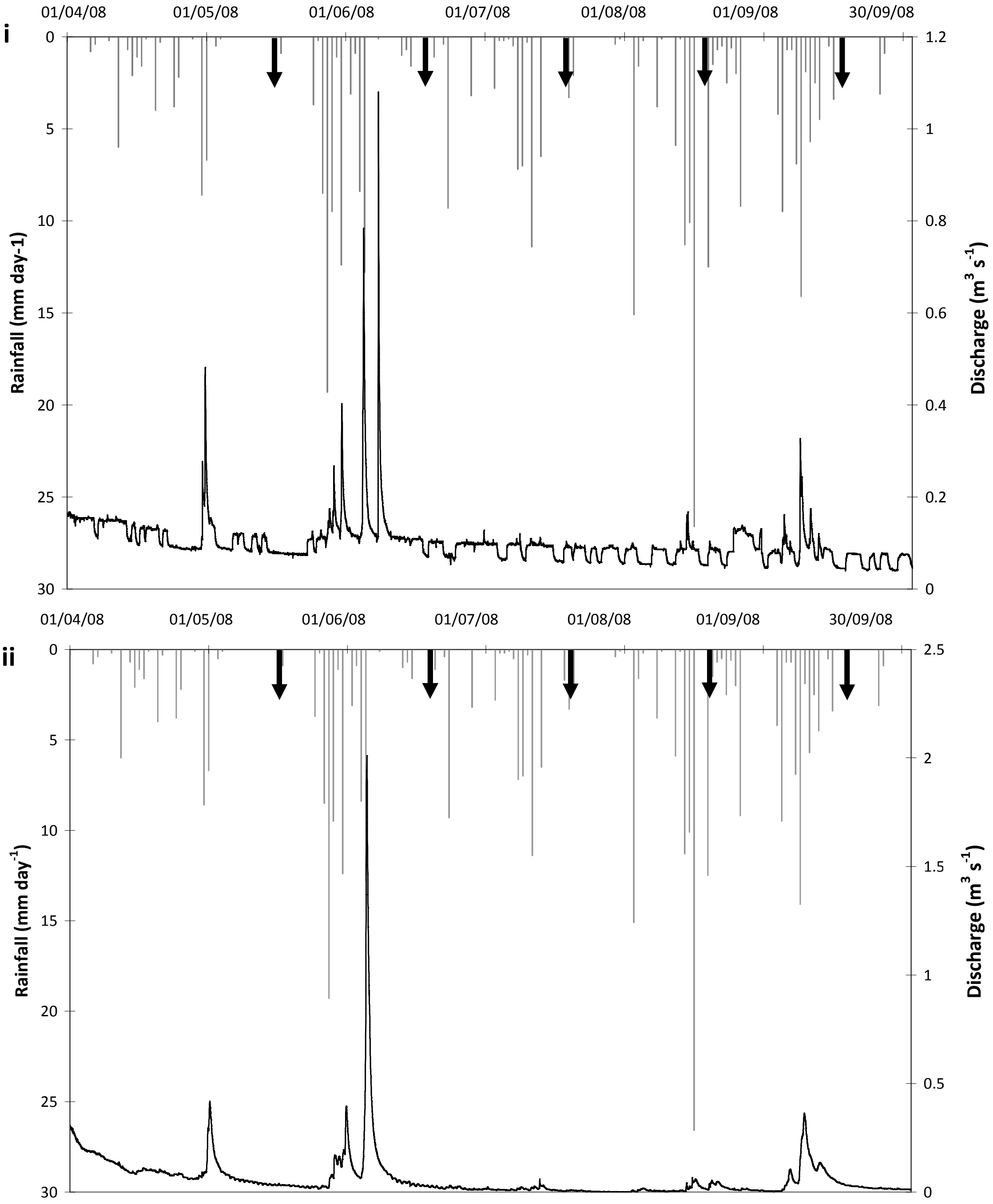


Figure 6.2: Daily rainfall at Carlby and 15-min resolution hydrographs for: i) the River West Glen at Little Bytham; ii) the River East Glen at Manthorpe (see Figure 3.6), April- September 2008. Arrows indicate sampling dates.

6.3.3 Flow duration analysis

Flow duration analysis was undertaken (Figure 6.3; Figure 6.4) and associated indices calculated (Table 6.3) to set the high and low flow conditions observed during the study year (water year ending 30th September 2008) in the context of the long-term (1981-2009) average conditions (see section 4.8.2).

On the East Glen at Manthorpe (~1 km downstream of site 4, Figure 3.6), the steep slope in the upper region of the FDC (Figure 6.3 (i)) and the low values of high-flow indices (Q_1, Q_5, Q_{10} and Q_{30}; Table 6.2) indicated that spate events were of a below-average magnitude and duration during the study year. However, the median value (Q_{50}), which provides a measure of 'average' discharge, was similar in 2007-08 to the LTA (Table 6.2). At the low-flow end of the curve (Figure 6.3(ii)), the rate at which

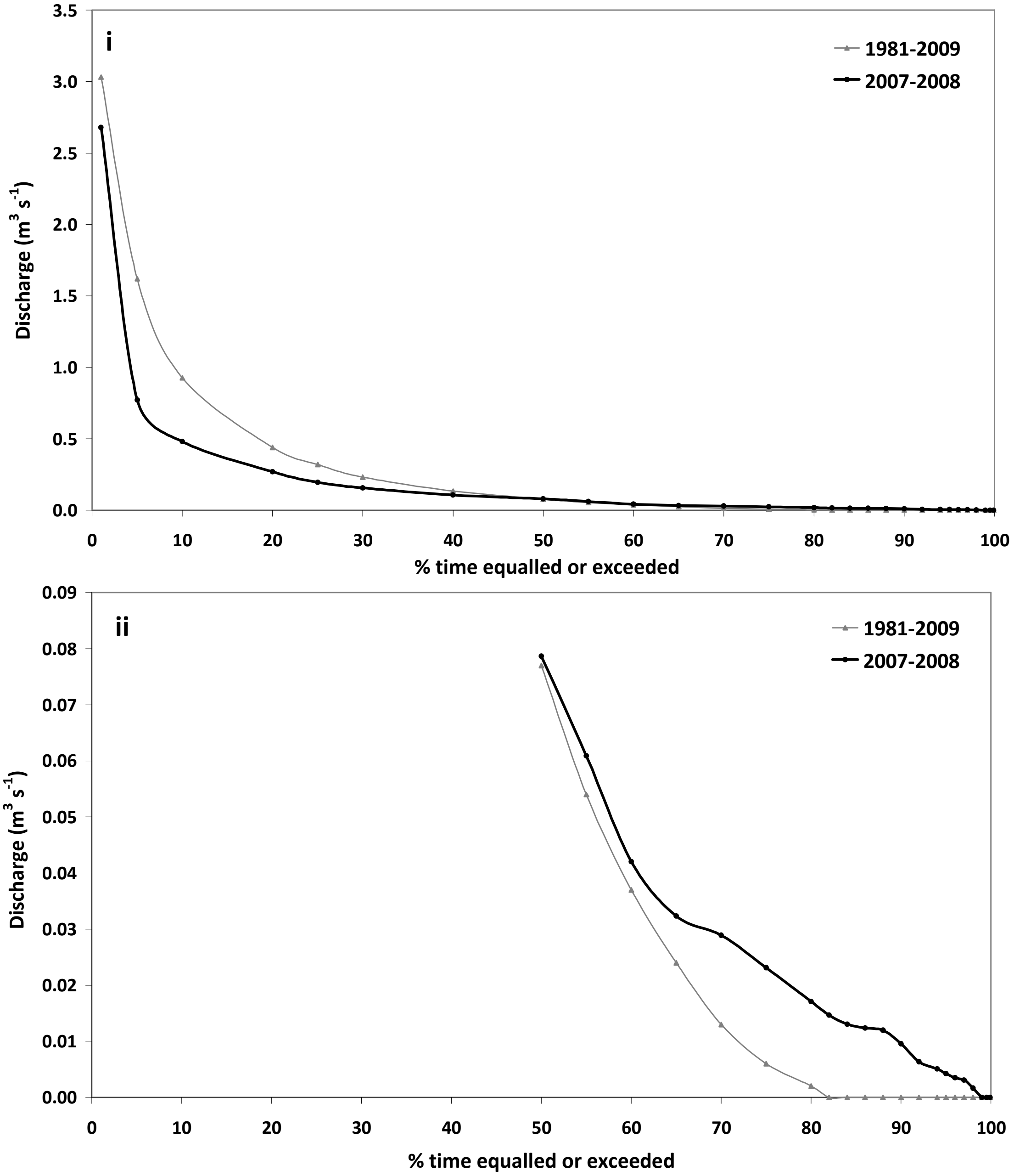


Figure 6.3: Flow duration curves for the River East Glen at Manthorpe. Mean daily discharge equalled or exceeded: i) 0-100 % time; ii) 0-50 % time.

discharge declined was more variable and slower than average, and zero-flow conditions occurred for an unusually short period (Q_{99} = 0 compared with Q_{90} = 0 for the LTA; Table 6.2).

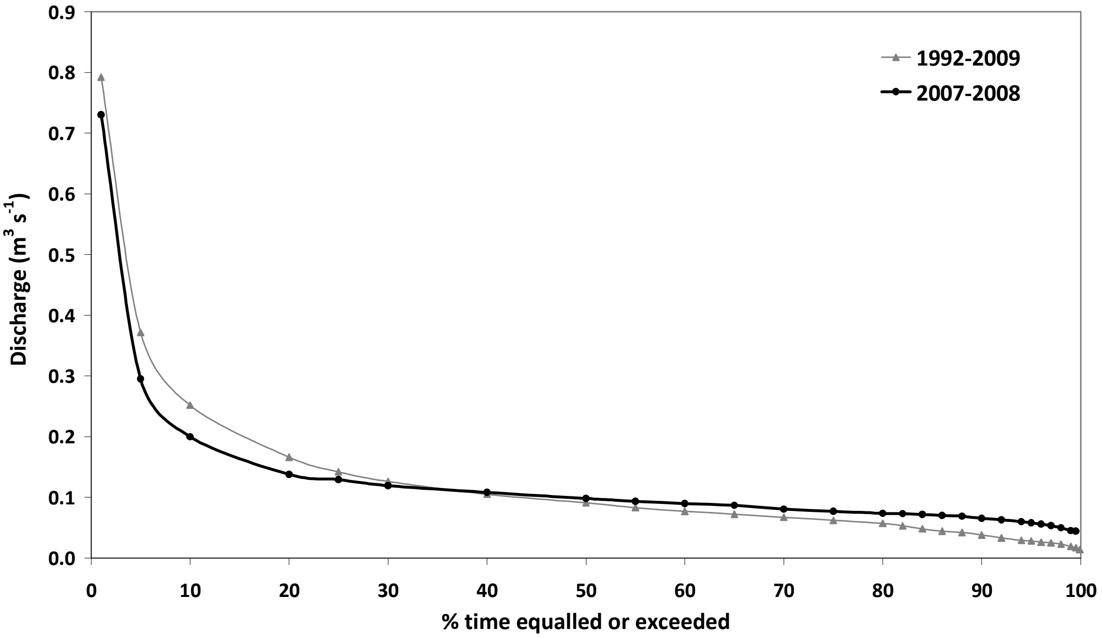


Figure 6.4: Flow duration curves for the River West Glen at Little Bytham. Mean daily discharge equalled or exceeded 1-100 % time.

Table 6.2: Indices derived from flow duration analysis, indicating discharge during the study year in comparison with the long term average

	Mean daily discharge (m^3 s^{-1})			
	Little Bytham (West Glen)		**Manthorpe (East Glen)**	
Index	**1992-2009**	**2007-2008**	**1981-2009**	**2007-2008**
Q_1	0.792	0.735	3.032	2.680
Q_5	0.372	0.295	1.620	0.770
Q_{10}	0.252	0.200	0.926	0.480
Q_{30}	0.126	0.119	0.231	0.156
Q_{50}	0.091	0.098	0.077	0.079
Q_{90}	0.038	0.065	0.000	0.010
Q_{95}	0.028	0.058	0.000	0.004
Q_{99}	0.019	0.045	0.000	0.000

On the West Glen at Little Bytham, the slope in the upper region of the FDC was only slightly steeper than is typical and median and high flow indices were similar to the LTA, whilst the low flow end of the curve and related indices remained above average (Figure 6.4; Table 6.2). However, all high flow indices were only slightly reduced in comparison with the LTA and, as is typical, flow never fell to zero. Comparison of the East and West Glen FDCs indicated that the East Glen experiences a flashier, more variable flow regime, including both streambed drying and higher magnitude spates (Figure 6.3; Figure 6.4).

6.4 Spatiotemporal variability in environmental conditions

Temporal changes in instream variables are considered (aim 1). First, the effects of discharge variability on the hydrological parameters measured instream are examined (objective 1), then the effects of these changes on submerged habitat availability are determined (objective 3). Associated changes in water quality parameters are also considered (objective 4), then principal components analysis is used to identify the main environmental gradients in the dataset (objective 5).

6.4.1 Variation in surface hydrology

In addition to the continuous discharge data obtained from the Environment Agency (Figure 6.2), water depth and mean flow velocity (at 0.6x depth) were measured each month at each sampling point, to determine the effects of discharge variability on the environment inhabited by the invertebrate community. Wetted width was also determined *post hoc* by application of depth measurements to cross-sectional channel profiles. Following description of temporal variability in these parameters, consequent changes in habitat availability are examined.

Surface water depth, flow velocity and wetted width

Spatially, mean depth was higher at both West Glen sites compared with the East Glen ($F_{1, 14} = 30.256$, $p < 0.001$; Table 6.4). Of the five sampling occasions, depth peaked in June and was lowest in September ($F_{1.827, 27.411} = 4.691$, $p = 0.020$; Figure 6.5(i); Table 6.3). The interaction with depth was significant for site ($F_{12, 48} = 8.487$, $p < 0.001$) and the overall pattern only reflected conditions at sites 1 ($F_{1.738, 5.213} = 6.726$, $p = 0.038$) and 3 ($F_{1.454, 4.363} = 6.837$, $p = 0.049$). At site 4, depth was considerably lower in July than in any other month ($F_{1.738, 5.213} = 6.726$, $p = 0.038$), whilst depth did not change significantly at site 2 (Figure 6.5(i)).

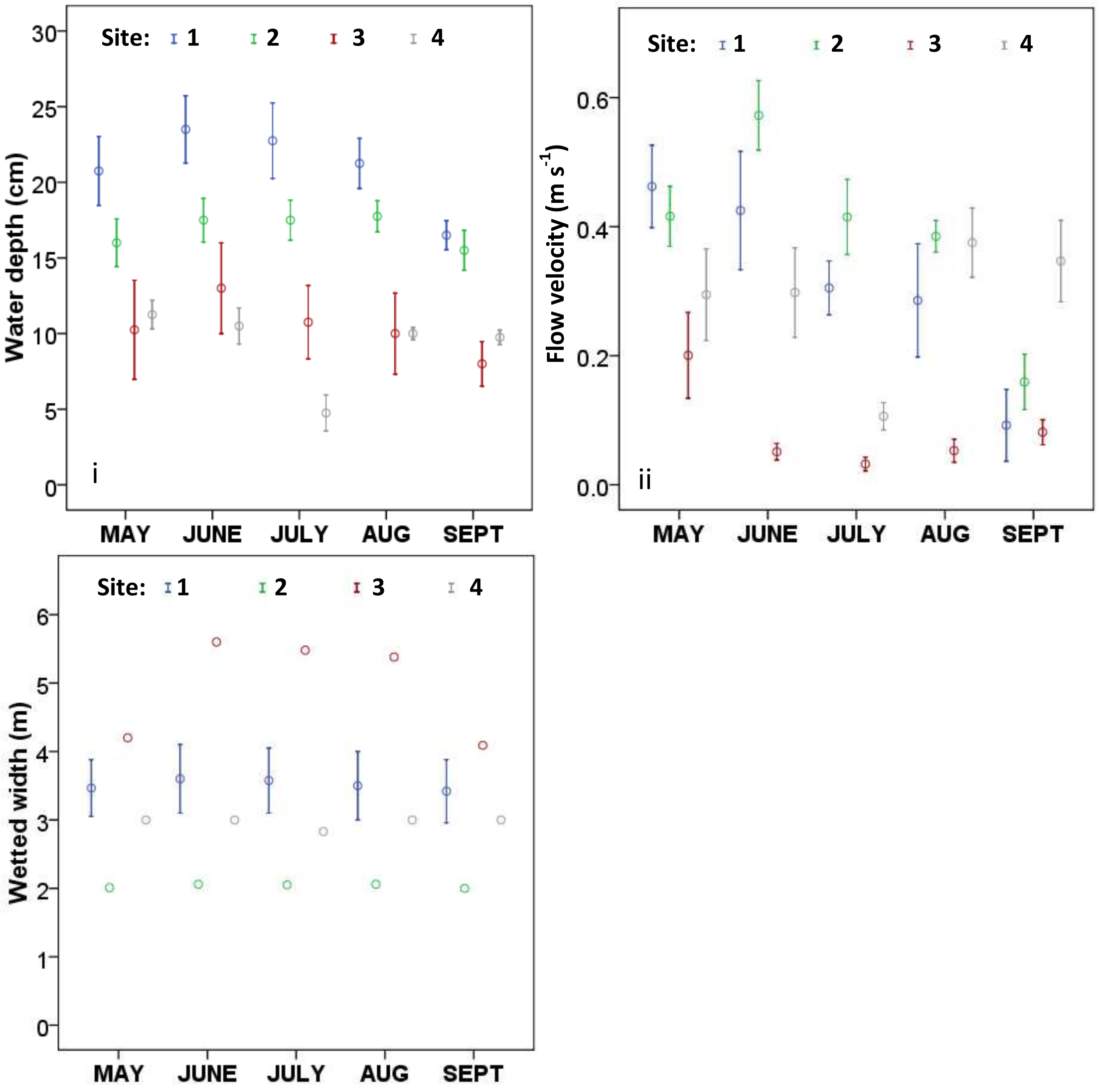


Figure 6.5: Mean ± 1 SE temporal change in hydrological variables: i) water depth; ii) mean flow velocity; iii) wetted width (replicates only available from site 1).

Considering all months, velocities were lower at site 3 than at any other site ($F_{3,\ 12}$ = 21.218, p <0.001; Table 6.4). Considering all sites, velocities peaked in May and June and were lowest in September ($F_{2.155,\ 32.328}$ = 5.516, p = 0.007; Table 6.3; Figure 6.5(ii)). The interaction with velocity was significant for site ($F_{12,\ 48}$ = 6.283, p <0.001) and the overall pattern of temporal change was only observed at site 1 ($F_{1.840,\ 5.521}$ = 6.121, p = 0.041). At site 2, the pattern was similar but with particularly high velocities in June ($F_{1.417,\ 4.251}$ = 8.594, p = 0.036); at site 3, velocities were higher in May than in any subsequent month, but temporal change was not significant; and at site 4, velocities were lower in July than in other months ($F_{1.801,\ 5.402}$ = 18.117, p = 0.004; Figure 6.5(ii)).

Mean wetted width was particularly high at site 3 and lowest at site 2 (Table 6.4; Figure 6.5(iii)). Mean values varied little between months, from 3.2 ± 0.4 m in May and September to 3.6 ± 0.60 m in June (Table 6.3; Figure 6.5(iii)), due to a combination of channel morphology (particularly on the West Glen; Appendix 8) and low variation in depth, which although significant, occurred within a range of <4.0 cm (Table 6.3). Considering individual sites, width at site 3 declined each month between June (5.6 m) and September (4.0 m), whilst at site 4 widths were very similar in all months (3.0 m) except July (2.8 m; Figure). Statistical analyses of these spatiotemporal changes in width were hampered by insufficient replicates.

Table 6.3: Temporal change in surface water hydrology of the River Glen, May to September 2008

Variable	**May**	**June**	**July**	**August**	**Sept**	**Temporal change***
Hydrological variables						
Surface water depth (cm)	14.6 ± 1.5	16.1 ± 1.6	13.9 ± 2.0	14.8 ± 1.5	12.4 ± 1.1	**
Mean flow velocity (m s^{-1})	0.34 ± 0.04	0.34 ± 0.06	0.26 ± 0.06	0.28 ± 0.04	0.17 ± 0.04	**
Wetted width (m)	3.2 ± 0.38	3.6 ± 0.60	3.5 ± 0.59	3.5 ± 0.56	3.2 ± 0.37	ns

Values presented as the mean ± 1 SE of all samples; *n* = 16 in all months for depth and velocity, *n* = 5 in all months for width. *One-way RM ANOVA tests; ** indicates *p* <0.01, ns indicates *p* >0.05.

Table 6.4: Spatial differences in surface water hydrology at River Glen sites 1-4.

Variable	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Spatial Change***
Surface water depth (cm)	21 ± 1.0	17 ± 0.6	10 ± 1.1	9 ± 0.6	**
Mean flow velocity (m s^{-1})	0.3 ± 0.04	0.4 ± 0.04	0.08 ± 0.02	0.3 ± 0.03	**
Wetted width (m)	3.5 ± 0.03	3.9 ± 0.04	5.0 ± 0.33	3.0 ± 0.03	ns

Values presented as the mean ± 1 SE of all samples; *n* = 20 at all sites for depth and velocity, *n* = 10 at site 1 and *n* = 5 at sites 2-4 for width. *Two-way RM ANOVA tests; ** indicates *p* <0.01, ns indicates *p* >0.05

6.4.2 Submerged habitat availability

Width and depth data were applied to cross-sectional channel profiles of each site to investigate the effect of discharge variability on submerged habitat availability (see section 4.5.2). The maximum extent of submerged benthic sediments recorded during the investigation was determined for each site using the cross-sectional levelling survey data and on-site observations regarding the extent of benthic sediments. The percentage of this maximum benthic habitat that was submerged and thus available for invertebrate inhabitation (% maximum submerged benthic habitat; % max. SBH) was then determined for each other month. At sites 1, 2 and 4, a single cross-section was representative of all sampling points, whilst two cross-sections were considered at site 3 due to variation in bed morphology (Appendix 8).

Table 6.5: Temporal change in extent of submerged benthic sediments as a percentage of the maximum recorded

	Submerged % of benthic sediments at site:				
Site	**1**	**2**	**3 (1)***	**3 (3)***	**4**
May	100	86.7	100	65.3	100
June	100	100	100	100	89.8
July	100	100	100	88.1	22.6
August	100	100	100	88.1	100
Sept	100	86.7	74.2	59.4	89.8

*** Bracketed numbers refer to the closest sampling point**

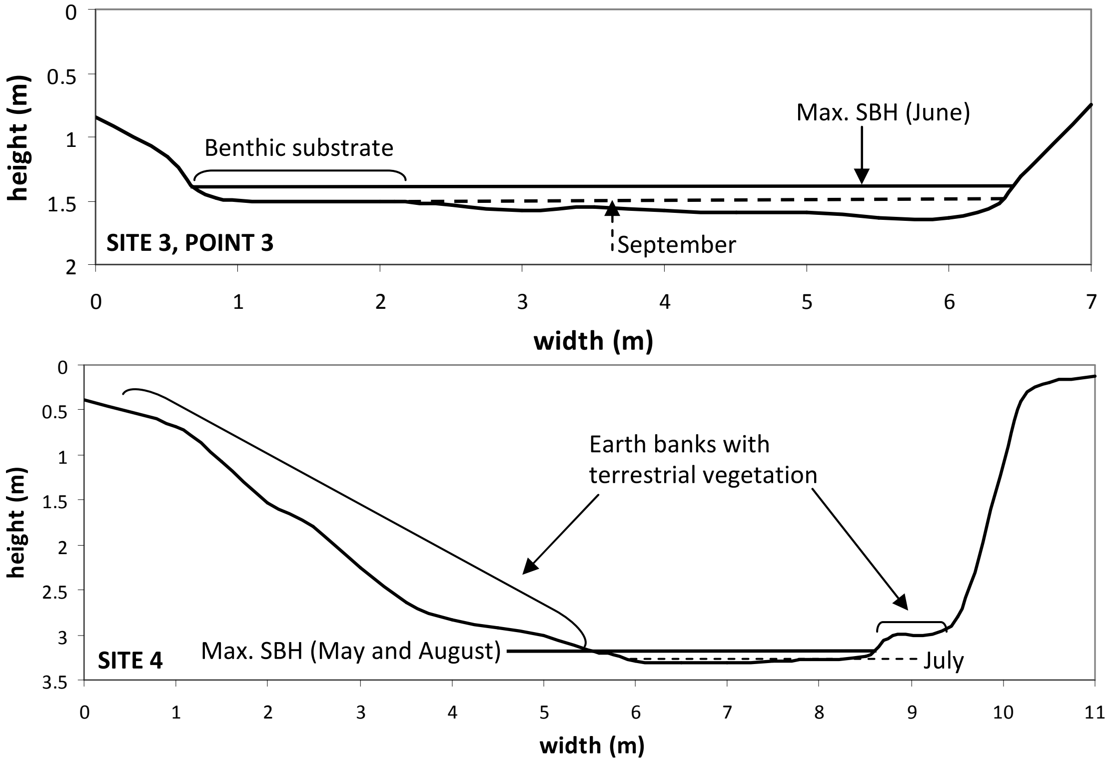


Figure 6.6: Cross-sectional profiles of sites 3 and 4, illustrating changes in the extent of submerged benthic sediments in relation to changes in water depth. Key: Max. SBH = maximum extent of submerged benthic habitat.

A trapezoidal channel shape at sites 1 and 2 resulted in maximum SBH being reached at a low depth, and the % max. SBH therefore remained similarly high in all months (Table 6.5). In contrast, the streambed of site 3 had a gentle cross-slope between steep banks (Figure 6.6) and the availability of submerged habitat was more responsive to changes in depth (Table 6.5). Whilst the streambed at site 4 could also be described as a gentle cross-slope between steep banks (Figure 6.6), depth at this site was very low in July (3 cm, compared to ≥9 cm in all other months), resulting in a dramatic decline in submerged habitat availability (Table 6.5; Figure 6.6).

6.4.3 Water quality

Temporal variability in water quality parameters that may have changed in response to variation in surface flow was examined; monthly mean values of all variables are presented in Table 6.6. Unless otherwise stated, values were comparable at all hyporheic depths, which were therefore pooled prior to analysis. Spatial differences were considered between tributaries, flow permanence groups and sites; for brevity, only significant patterns are described (also see Table 6.7). Also for brevity, non-significant results are not always stated; p >0.05 in all cases.

Dissolved oxygen

DO readings were not obtained at sites 2 and 4 in June or September; these months were therefore excluded from analysis unless otherwise specified. Both concentration and saturation were determined (and are detailed in Tables 6.6 and 6.7), but these measures followed the same spatial and temporal patterns and therefore only % saturation is described in detail.

DO levels in surface water were considerably lower at East Glen sites compared with the West Glen ($F_{1, 14}$ = 11.382, p = 0.005; Table 6.7). DO availability was higher in May, June and September, and relatively low in July and August ($F_{1.234, 18.510}$ = 5.614, p = 0.023; Table 6.6; Figure 6.7(i)). The interaction with site was significant ($F_{3.604, 14.415}$ = 12.033, p <0.001), and June and September data could be included in analysis of sites 1 and 3. At site 1, DO peaked in June and was lowest in July and August ($F_{4, 12}$ = 161.020, p = 0.001); similarly, at site 2, DO was higher in May compared with July and August ($F_{2, 6}$ = 190.176, p <0.001); at site 3, DO was particularly low in August and peaked in September ($F_{1.333, 3.998}$ = 19.419, p = 0.010); and at site 4, DO availability was lowest in July but temporal change was not significant (Figure 6.7(i)).

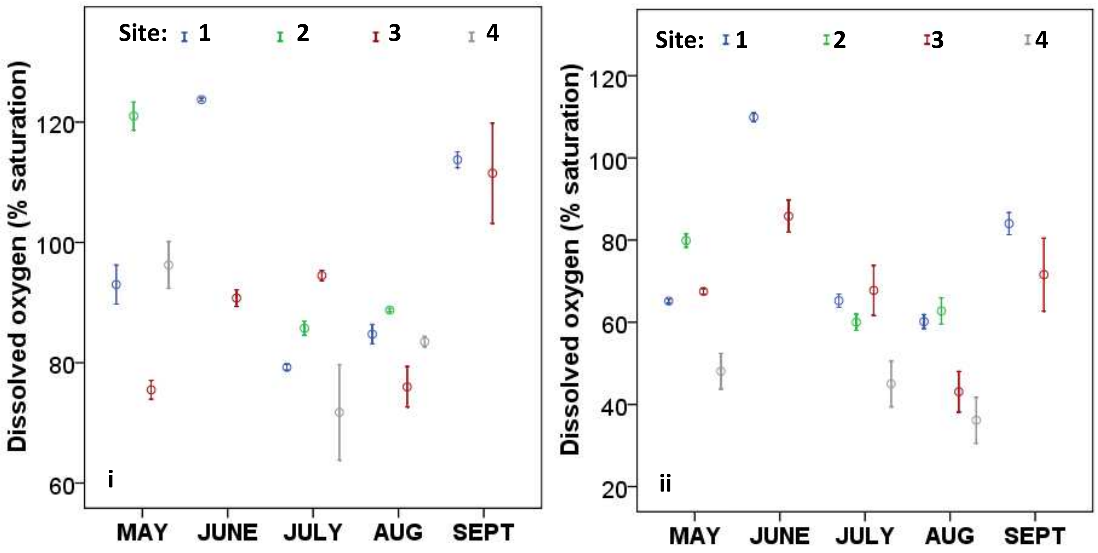


Figure 6.7: Mean ± 1 SE temporal change in dissolved oxygen (% saturation): i) surface water; ii) hyporheic water. No data is available for site 2 or site 4 in June or September.

Mean DO values were significantly lower in hyporheic water (57.6 ± 1.5 %) compared with surface water (87.5 ± 2.0 %; $F_{1, 58} = 72.647$, $p < 0.001$) and decreased slightly with increasing hyporheic depth. Spatially, hyporheic DO levels were particularly low at site 4 ($F_{3, 40} = 13.142$, $p < 0.001$; Table 6.7). Hyporheic DO peaked in June, was low in July and particularly low in August ($F_{2, 86} = 17.019$, $p < 0.001$; Table 6.6; Figure 6.7(ii)).

The interaction with site was significant for hyporheic DO ($F_{6, 80} = 4.412$, $p = 0.001$), and data were available for all months at sites 1 and 3. At site 1, DO was particularly high in June and was lowest in August ($F_{4, 44} = 158.365$, $p < 0.001$); a similar pattern was seen at site 3, although the June peak was less prominent and the August dip more pronounced ($F_{1.937, 21.306} = 13.322$, $p < 0.001$); at site 2, DO values were considerably higher in May compared with July and August ($F_{2, 14} = 18.336$, $p < 0.001$); and at site 4, DO declined gradually between May and August and was very low in the latter month (mean 36.2 ± 5.6 %), but temporal change was of only marginal significance ($F_{2, 22} = 2.953$, $p = 0.073$).

Water temperature

Surface water temperatures ranged between 11.3 ^{o}C at sites 1 and 3 in May to 16.2 ^{o}C at site 3 in July, whilst mean values were particularly high at site 4 ($F_{3, 12}$ =

131.466, *p* <0.001; Table 6.7). Temporally, mean temperatures were lowest in May then increased each month to a July/August peak ($F_{1.978, 29.671}$ = 39.171, *p* <0.001; Table 6.6; Figure 6.8(i)). The interaction with temperature was significant for site ($F_{12, 48}$ = 209.906, *p* <0.001), with slight variations on the overall pattern observed at sites 2, 3 and 4 ($F_{\geq 128.535}$, *p* <0.001). At site 1, lowest temperatures also occurred in May, but this was followed by a June peak ($F_{1.534, 4.601}$ = 400.039, *p* <0.001).

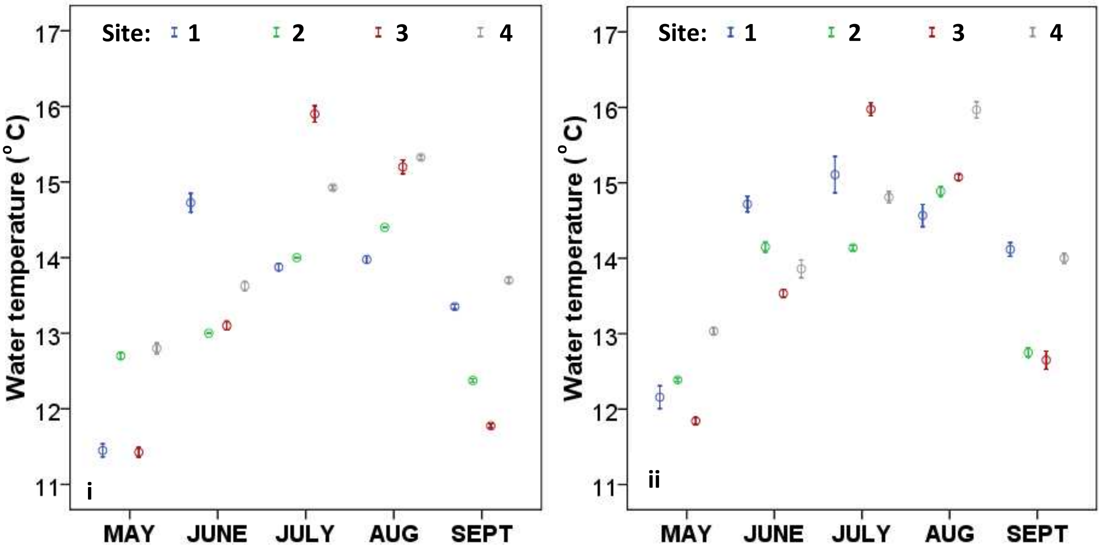


Figure 6.8: Mean ± 1 SE temporal change in water temperature (°C): i) surface water; ii) hyporheic water.

Mean temperature was higher in hyporheic water (14.0 ± 0.08 °C) than in surface water (13.6 ± 0.14°C; $F_{1, 58}$ = 19.902, *p* = 0.013), but similar at all hyporheic depths. Significant differences in hyporheic temperature were observed between sites, with similarly high temperatures at sites 1 and 4, and significantly lower temperatures at sites 2 and 3 ($F_{3, 40}$ = 17.674, *p* <0.001; Table 6.7). Considering all sites, patterns of temporal variability in hyporheic temperature reflected those reported for surface water and were highly significant ($F_{2.559, 110.042}$ = 150.053, *p* <0.001; Table 6.6; Figure 6.8(ii)). Whilst the interaction with water temperature was significant for site ($F_{8.589, 114.524}$ = 49.044, *p* <0.001), site-specific patterns of change differed only slightly in statistical significance and in the timing of peak values.

Particulate organic carbon

Surface water POC concentrations were lowest at site 1 and higher at all other sites, particularly site 4, but spatial variation was not significant ($F_{3, 8} = 0.675$, $p = 0.591$; Table 6.7). Considering all sites, surface POC concentrations decreased and became less variable between July and September ($F_{2, 6} = 5.427$, $p = 0.045$; Figure 6.9(i); Table 6.6). The interaction with POC was not significant for any spatial parameter and monthly declines occurred at all sites.

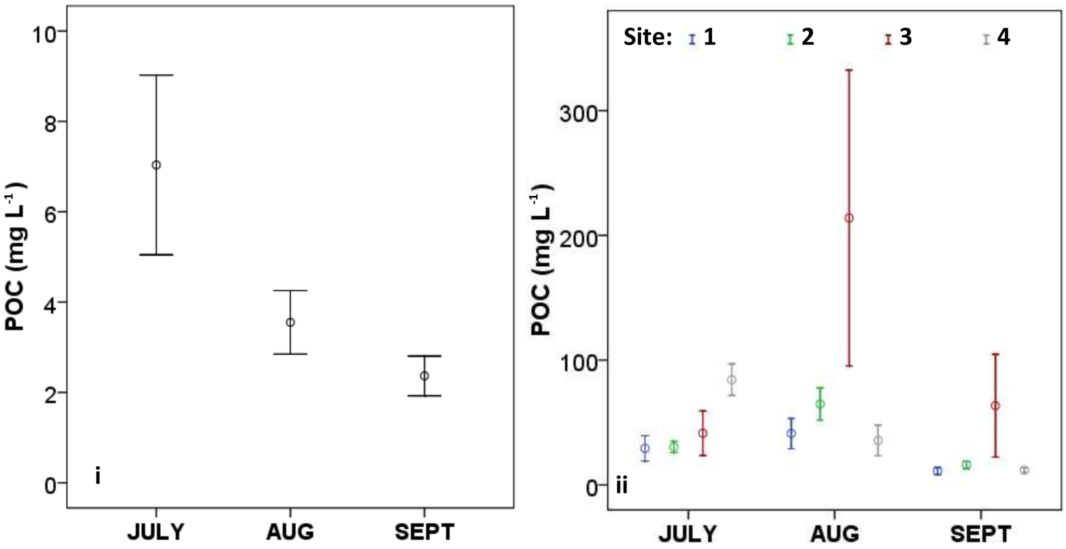


Figure 6.9: Mean ± 1 SE temporal change in particulate organic carbon concentrations (mg L^{-1}): i) surface water (pooled data); ii) hyporheic water.

POC concentrations in surface and hyporheic water could not be compared due to different sampling techniques, but values were similar at all hyporheic depths. Hyporheic concentrations were very high at site 3 compared to all other sites but values were statistically comparable ($F_{3, 7} = 1.231$, $p = 0.368$; Table 6.7). Overall, POC concentrations were high (but very variable) in August and lowest in September ($F_{1.165, 11.651} = 8.977$, $p = 0.009$; Figure 6.9(ii); Table 6.6), with a significant interaction being observed with site ($F_{6, 14} = 3.884$, $p = 0.017$). Temporal change was not, however, significant at any individual site.

Fine sediment

Surface water fine sediment concentrations were considerably higher at site 3 compared with all other sites, but spatial variability was not significant ($F_{3, 8} = 1.090$,

$p = 0.407$; Table 6.7). Concentrations were high and variable in August, due in part to a site 3 outlier, and lowest and in September; temporal change was not significant ($F_{2,6} = 0.682$, $p = 0.541$; Table 6.6; Figure 6.10(i)). Interactions with fine sediment were not significant for any spatial parameter and insufficient data were available for site-specific analyses. Concentrations in surface and hyporheic water were not compared due to different sampling techniques, but were comparable at all hyporheic depths. As in surface water, hyporheic concentrations were much higher at site 3 than at other sites but this was not significant ($F_{3,8} = 1.185$, $p = 0.375$; Table 6.7); neither was temporal change significant (Table 6.6; Figure 6.10(ii)).

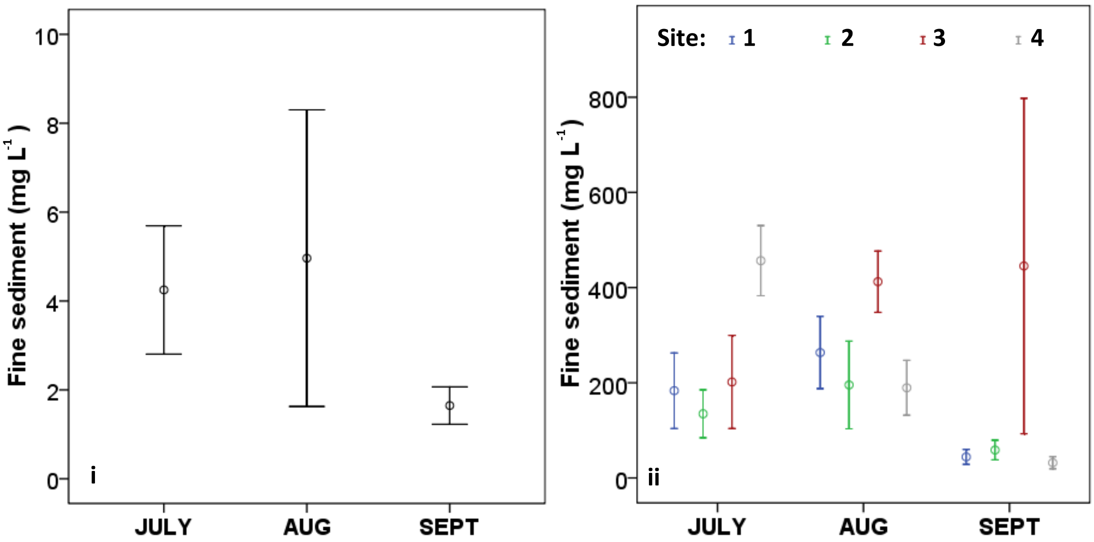


Figure 6.10: Mean ± 1 SE temporal change in fine sediment concentrations (mg L^{-1}): i) surface water; ii) hyporheic water (a September site 3 outlier has been normalised from 2898 mg L^{-1} to be in line with other values).

Nitrate and phosphate

Mean surface water nitrate concentrations were considerably higher at West Glen sites compared with the East Glen ($F_{1,2} = 250.416$, $p = 0.004$); this pattern was also seen in hyporheic water (Table 6.7). Surface and hyporheic nitrate concentrations were very similar ($F_{3,7} = 0.038$, $p = 0.989$). However, whilst temporal change was not significant overall or at any individual site in surface water, in hyporheic water concentrations were lowest in June then peaked in August and remained high in September ($F_{4,28} = 8.525$, $p < 0.001$; Table 6.6).

Surface water phosphate concentrations were also higher on the West Glen compared with East Glen ($F_{1,2} = 230.677$, $p = 0.004$; Table 6.7); temporal change was not significant (Table 6.6). Concentrations were comparable in surface water and at all hyporheic depths ($F_{3,11} = 0.264$, $p = 0.850$). Spatial variations in hyporheic phosphate concentrations were equivalent to those reported for surface water. Considering all sites, mean hyporheic concentrations were lowest in May then increased to a peak in August ($F_{4,40} = 10.491$, $p < 0.001$; Table 6.6); patterns varied spatially but temporal change was not significant at any individual site.

Conductivity and pH

Conductivity was high on the River Glen (overall mean 818 ± 5.4 µS cm^{-1}). Whilst changes in mean values were spatiotemporally significant, these changes were too minor to be of ecological relevance and they are therefore not examined in detail (but see Tables 6.6 and 6.7). However, it is worth noting that values were slightly higher in hyporheic water (833 ± 3.8 µS cm^{-1}) compared with surface water (818 ± 5.4 µS cm^{-1}), and that values were particularly high at site 3 (surface water mean 879 ± 6.6 µS cm^{-1}; hyporheic mean 911 ± 7.6 µS cm^{-1}; peak 1066 µS cm^{-1}; Table 6.7).

Significant spatial and temporal variability was also observed in pH, but mean values were moderate in all months in both surface and hyporheic water (Table 6.6 and 6.7); detailed analysis of this variable is therefore not required. However, it is of note that numerous particularly high values (8.7-9.1) were recorded across all sites in both surface and hyporheic water in July (Table 6.6).

Table 6.6: Temporal change in physicochemical measures of surface and hyporheic water in the River Glen, May to September 2008

Variable	Surface or hyporheic	May	June	July	August	Sept	Temporal change
DO (mg L^{-1})	Surface	9.6 ± 0.59	9.0 ± 0.77	7.3 ± 0.24	7.1 ± 0.16	9.4 ± 0.49	**
	Hyporheic	5.43 ± 0.25	8.03 ± 0.38	4.55 ± 0.29	3.49 ± 0.27	5.45 ± 0.43	**
DO (% saturation)	Surface	96.4 ± 4.4	107.3 ± 6.3	82.8 ± 2.8	83.3 ± 1.5	112.6 ± 3.9	*
	Hyporheic	63.8 ± 2.0	97.9 ± 3.2	59.5 ± 2.6	49.4 ± 2.7	77.8 ± 4.7	**
Water temperature (^{o}C)	Surface	12.1 ± 0.17	13.6 ± 0.18	14.7 ± 0.21	14.7 ± 0.15	12.8 ± 0.20	**
	Hyporheic	12.4 ± 0.08	14.1 ± 0.08	15.1 ± 0.12	15.1 ± 0.10	13.4 ± 0.11	**
Fine sediment (mg L^{-1})	Surface	-	-	4.2 ± 1.4	5.0 ± 3.3	1.6 ± 0.4	ns
	Hyporheic	-	-	240 ± 50	480 ± 230	450 ± 90	ns
POC (mg L^{-1})	Surface	-	-	7.0 ± 2.0	3.6 ± 0.7	2.4 ± 0.4	*
	Hyporheic	-	-	47.8 ± 9	91.1 ± 37	26.5 ± 12	**
Nitrate (mg L^{-1})	Surface	3.2 ± 1.63	1.7 ± 0.88	2.7 ± 1.25	4.3 ± 1.02	4.4 ± 1.01	ns
	Hyporheic	3.2 ± 0.88	1.6 ± 0.70	3.1 ± 0.56	5.0 ± 1.39	4.6 ± 0.65	**
Phosphate (mg L^{-1})	Surface	0.49 ± 0.21	0.42 ± 0.15	0.62 ± 0.20	0.76 ± 0.09	0.77 ± 0.30	ns
	Hyporheic	0.21 ± 0.07	0.38 ± 0.10	0.65 ± 0.09	0.71 ± 0.06	0.61 ± 0.14	**
pH	Surface	8.1 ± 0.03	8.2 ± 0.02	8.3 ± 0.19	7.8 ± 0.06	8.1 ± 0.02	*
	Hyporheic	8.0 ± 0.04	8.1 ± 0.05	8.3 ± 0.07	7.5 ± 0.07	8.0 ± 0.03	*
Conductivity (μS cm^{-1})	Surface	816 ± 12.6	812 ± 12.9	794 ± 12.6	818 ± 6.9	847 ± 11.4	**
	Hyporheic	845 ± 9.5	847 ± 9.4	804 ± 10.5	830 ± 6.6	864 ± 8.9	**

Values presented as the mean ± 1 SE of all samples. In each month, *n* = 16 for surface water and *n* = 44 for hyporheic water (comprising *n* = 16 from 10 cm and 20 cm, *n* = 12 from 30cm), with the following exceptions: surface DO (mg L^{-1} and % saturation), *n* = 8 in June and *n* = 7 in September; hyporheic DO (mg L^{-1} and % saturation), *n* = 24 in June and September; hyporheic conductivity, *n* = 36 in all months; hyporheic nitrate, *n* = 8 in all months; hyporheic phosphate, POC and fine sediment, *n* = 11 in all months. Temporal change analysed using one-way RM ANOVA: * indicates p <0 .05; ** indicates p <0.01; ns indicates p >0.05. Key: DO = dissolved oxygen; POC = particulate organic carbon.

Table 6.7: Spatial differences in physicochemical measures of surface and hyporheic water at River Glen sites 1-4.

Variable	Surface or hyporheic	Site 1	Site 2	Site 3	Site 4	Spatial change?
DO (mg L^{-1})	Surface	9.0 ± 0.5	8.9 ± 0.6	7.5 ± 0.3	7.6 ± 0.4	**
	Hyporheic	6.2 ± 0.2	5.6 ± 0.3	5.2 ± 0.3	2.8 ± 0.3	**
DO (% saturation)	Surface	86 ± 2.0	99 ± 4.9	82 ± 2.9	84 ± 4.0	**
	Hyporheic	77 ± 2.5	68 ± 2.3	67 ± 3.0	43 ± 3.0	**
Temperature (°C)	Surface	13.5 ± 0.3	13.3 ± 0.2	13.5 ± 0.4	14.1 ± 0.2	**
	Hyporheic	14.1 ± 0.2	13.7 ± 0.2	13.8 ± 0.2	14.3 ± 0.1	**
Fine sediment (mg L^{-1})	Surface	1.84 ± 0.3	3.53 ± 2.4	7.10 ± 4.0	1.99 ± 0.8	ns
	Hyporheic	163 ± 45	129 ± 37	640 ± 314	226 ± 68	ns
POC (mg L^{-1})	Surface	2.36 ± 0.6	4.32 ± 0.8	4.89 ± 1.0	5.70 ± 3.5	ns
	Hyporheic	26.1 ± 5.8	34.6 ± 8.7	99.2 ± 41.3	41.1 ± 10.9	ns
Nitrate (mg L^{-1})	Surface	4.8 ± 0.8	5.4 ± 0.6	1.3 ± 0.4	1.6 ± 0.8	**
	Hyporheic	6.2 ± 1.0	5.2 ± 0.4	1.7 ± 0.4	1.8 ± 0.4	**
Phosphate (mg L^{-1})	Surface	0.79 ± 0.1	0.85 ± 0.2	0.38 ± 0.1	0.41 ± 0.2	**
	Hyporheic	0.65 ± 0.09	0.86 ± 0.07	0.37 ± 0.08	0.28 ± 0.06	**
pH	Surface	7.9 ± 0.08	8.4 ± 0.08	8.1 ± 0.03	8.1 ± 0.12	*
	Hyporheic	8.1 ± 0.03	8.2 ± 0.08	8.0 ± 0.03	7.7 ± 0.07	**
Conductivity (µS cm^{-1})	Surface	802 ± 5.5	806 ± 7.0	879 ± 6.6	783 ± 8.7	**
	Hyporheic	815 ± 2.5	803 ± 4.6	903 ± 5.1	795 ± 4.8	**

Values given as mean ± 1 SE of all samples. Surface water: *n* = 20 for each site; hyporheic water: *n* = 60 at sites 1, 3 and 4 (⅓ from 10, 20, 30 cm) and *n* = 40 at site 2 (½ from 10, 20 cm), with the following exceptions: surface DO (mg L^{-1} & %), *n* = 12 for site 2 & 4; hyporheic DO (mg L^{-1}& %), *n* = 24 for site 2, *n* = 36 for site 4; surface POC and fine sediment, *n* = 3; hyporheic POC and fine sediment, *n* = 9; surface nitrate and phosphate, *n* = 5; hyporheic nitrate and phosphate, *n* = 9 for sites 1, 3 and 4 and *n* = 6 for site 2. Key: DO = dissolved oxygen; POC = particulate organic carbon.

6.4.4 Principal Components Analysis

PCA was used to investigate spatiotemporal variation in environmental conditions in both the surface stream and the hyporheic zone, and to identify the major gradients underlying this variation.

Surface water

PCA ordinations are presented both by month and by site (Figure 6.11(i) and (ii)). The first principal component (PC1) explained 32.6 % of the variance and was strongly correlated with conductivity (Pearson correlation coefficient (PCC) = 0.797, p <0.001) and flow velocity (PCC = -0.804, p <0.001). PC2 explained a further 28.3 % of the variance had significant positive correlations with temperature (PCC = 0.632, p <0.001) and pH (PCC = 0.552, p <0.001). Water depth was similarly correlated with both PC1 (PCC = -0.551, p <0.001) and PC2 (PCC = -0.558, p <0.001).

Although considerable overlap was observed between samples from all months (Figure 6.11(i)), temporal change was significant on both PC1 ($F_{4, 75} = 3.199$, $p = 0.018$) and PC2 ($F_{4, 75} = 6.462$, $p < 0.001$). PC1 scores were similar between May and August but particularly high in September due to high conductivity and low flow velocities. PC2 scores were particularly high in July but comparable in all other months, reflecting high temperatures and pH at site 4 in July (Figure 6.11(i) and (ii)). Comparison of Figures 6.11(i) and (ii) indicated that environmental conditions were influenced more strongly by spatial variability than temporal variability, and these spatial differences were primarily site-specific. PC1 scores were particularly high at site 3 ($p < 0.001$ compared with other sites), reflecting high conductivity and slow flow velocities. PC2 scores were significantly higher at site 4 than at any other site ($p \leq 0.016$), due to high temperatures and high pH. No other significant differences were recorded between sites, and some sites (particularly adjacent sites 1 and 2 on the West Glen) showed complete overlap (Figure 6.11(ii)).

Hyporheic water

Both PC1 and PC2 had significant correlations with all variables ($p \leq 0.036$), although many relationships were weak (PCC ≥0.148). PC1 explained 46.7 % of the variance and was strongly correlated with both oxygen parameters (PCC = -0.876-0.880, $p < 0.001$). PC2 explained a further 17.1 % of the variance and was strongly correlated with conductivity (PCC = 0.808, $p < 0.001$) and temperature (PCC = -0.680, $p < 0.001$; Figure 6.12).

Despite considerable overlap between months, temporal change was significant on both PC1 ($F_{4, 195} = 18.524$, $p < 0.001$) and PC2 ($F_{4, 195} = 22.390$, $p < 0.001$). PC1 scores were similar and low in May and June, reflecting relatively high DO availability, increased in July then peaked in August as DO availability declined. PC2 scores were high in May, June, and September, reflecting lower temperatures and higher conductivity in these months. PC2 scores were particularly low in July, reflecting low conductivities and high temperatures (Figure 6.12(i)).

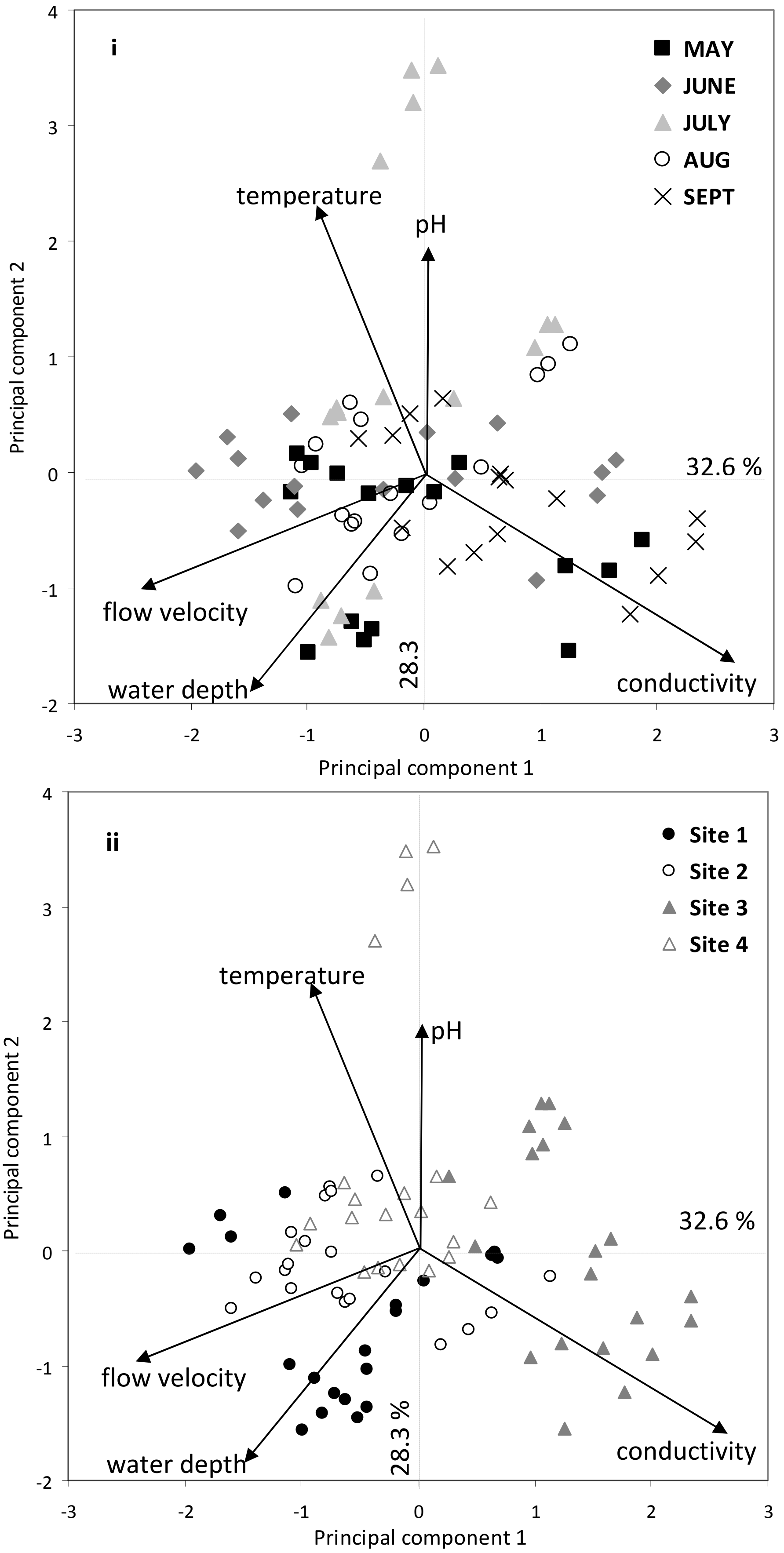


Figure 6.11: Principal components analysis of surface water data: i) temporal variability; ii) spatial variability in environmental conditions. Key: circles = West Glen; triangles = East Glen.

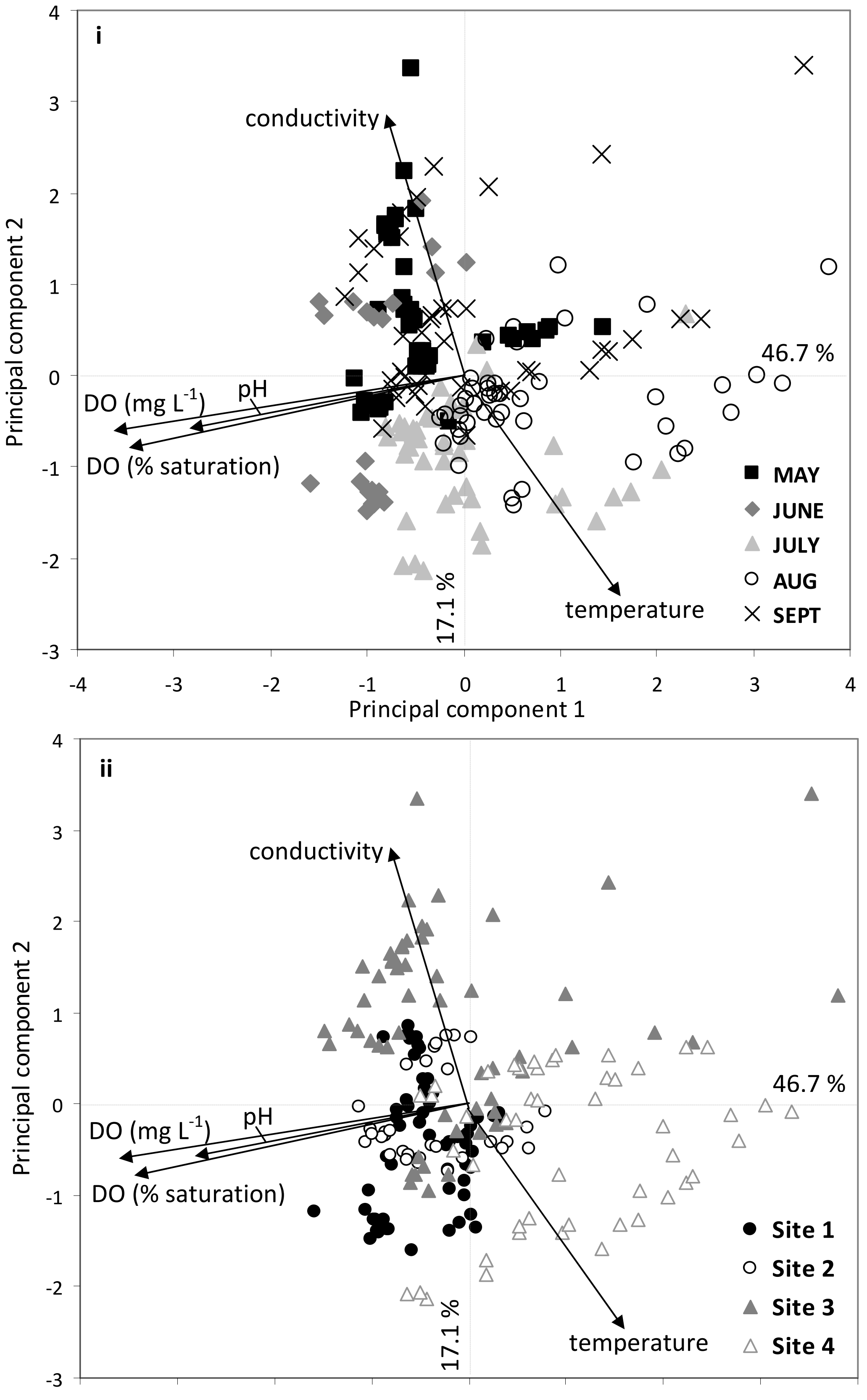


Figure 6.12: Principal components analysis of hyporheic water data: i) temporal variability; ii) spatial variability in environmental conditions. Key: circles = West Glen; triangles = East Glen.

Comparison of Figures 6.12 (i) and (ii) indicated that much environmental variation was explained by site-specific differences. PC1 scores were similar at sites 1, 2 and 3 but significantly higher at site 4 ($F_{3, 196}$ = 34.848, p <0.001), reflecting the low DO concentrations recorded at the latter site (Figure 6.12(ii)). PC2 scores were similar at sites 1, 2 and 4 and higher at site 3 ($F_{3, 196}$ = 37.026, p <0.001), reflecting high conductivity and temperature values recorded at site 3. West Glen sites 1 and 2 formed a tight, partly overlapping cluster at the centre of the ordination, whilst East Glen sites were separated on PC2, and had highly variable scores on PC1, reflecting the wide range of DO values recorded at these sites (Figure 6.12(ii)).

6.5 Spatiotemporal variability in the benthic invertebrate community

Spatial and temporal variability in the composition of the invertebrate community inhabiting the benthic sediments is examined to address two aims: firstly, to analyse changes in the abundance of taxa with the potential to increase the strength of biotic interactions (aim 1, objective 6), and secondly, to consider changes in community composition arising as a result of variation in hydrological or hydrologically-mediated (biotic and abiotic) variables (aim 2, objectives 1 and 2).

Community description

A total of 54,532 invertebrates were recorded from 80 Surber samples taken from the benthic sediments. The community comprised members of 103 taxa, including 72 identified to species level and 31 higher taxa that may have included multiple representatives.

6.5.1 Detrended correspondence analysis

DCA was conducted to investigate spatial and temporal variability in community composition (Figures 6.13 and 6.14). Axis 1 explained 18.5 % of the variation in the species data and had significant (p <0.05) correlations with the abundance of just two taxa, *Oulimnius* spp. (larvae) and *G. pulex*. Axis 2 explained a further 11.8 % of the variance and had highly significant (p <0.01) correlations with *P. antipodarum*, Sphaeriidae, *E. octoculata*, *Oulimnius* (larvae), *S. ignita* and *Baetis* spp. abundance.

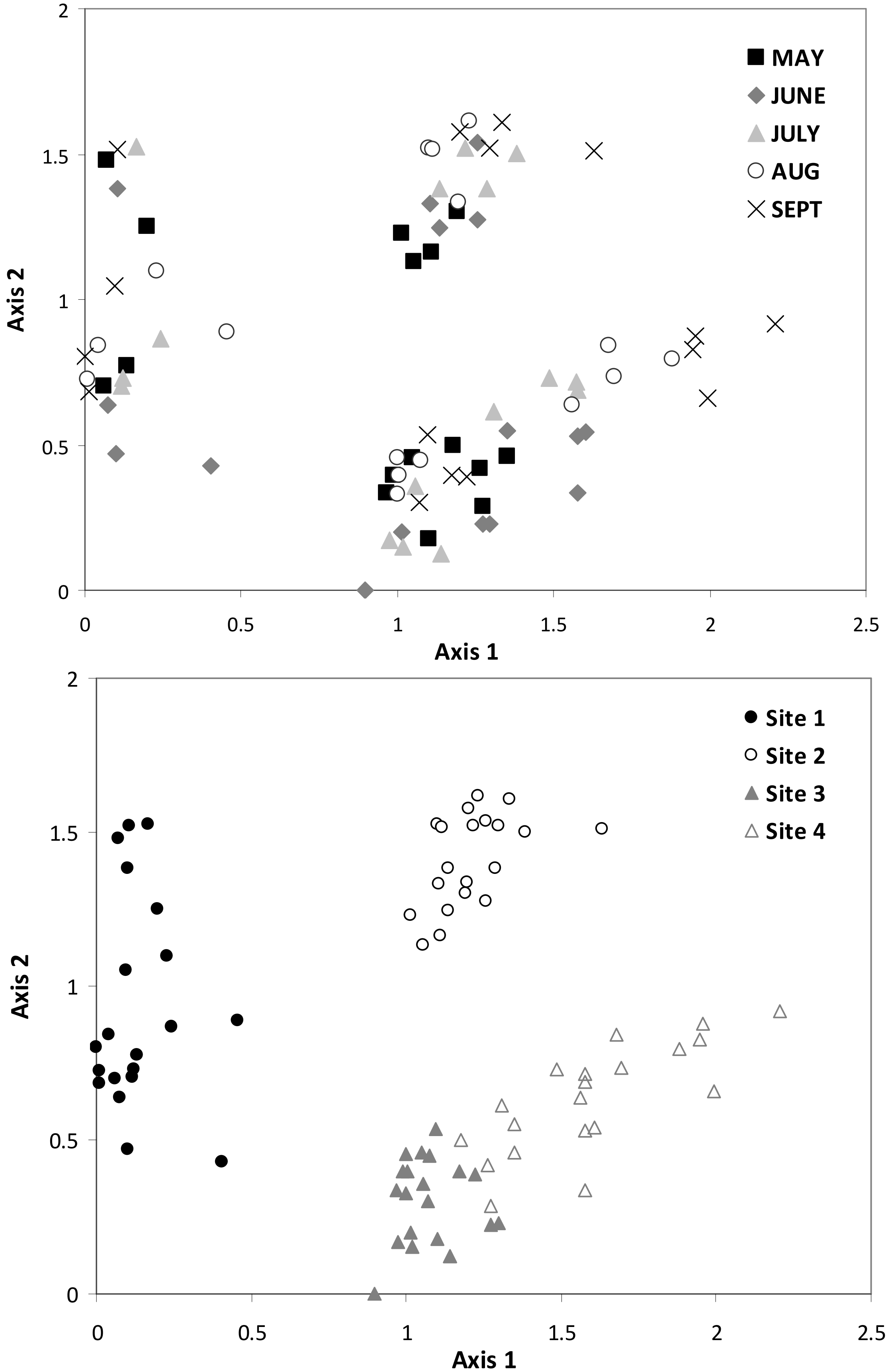


Figure 6.13: Detrended correspondence analysis sample plot of benthic community data: i) temporal variability; ii) spatial variability. Key: circles = West Glen; triangles = East Glen.

Temporal variability

Considering all sites, temporal change in community composition was demonstrably not responsible for the observed clustering, with samples from all months present in each cluster (Figure 6.13(i)). However, when site-specific differences were taken into account using RM ANOVA, temporal change was significant on both axis 1 ($F_{2.083, 31.244} = 5.652$, $p = 0.007$) and axis 2 ($F_{4, 60} = 9.345$, $p < 0.001$). Axis 1 scores were lowest in May, moderate between June and August then peaked in September; axis 2 scores tended to increased in each month, although a June dip was observed at sites 1 and 2 (Figure 6.13(i)).

Positioning of some taxa on the species plot (Figure 6.14) reflected their temporal occurrence, for example Simuliidae were particularly abundant in May and plotted in the negative quadrant of axis 1. In other cases, temporal associations were less apparent, for example the Sphaeriidae had a similar axis 1 score to the Simuliidae despite peak abundance in August. Ubiquitous taxa, (Chironomidae, Oligochaeta), plotted at the centre of the species ordination, regardless of temporal change in their occurrence (Figure 6.14).

Spatial variability

The clustering observed in the sample plots reflected spatial variability between sites, with West Glen sites 1 and 2 forming two distinct groups and East Glen sites 3 and 4 plotting with slight overlap as a single group (Figure 6.13(ii)). West Glen sites had higher axis 2 scores than East Glen sites ($F_{1, 14} = 26.531$, $p < 0.001$), whilst intermittent sites (2, 4) had higher axis 1 scores than perennial sites (1, 3; $F_{1, 14} = 17.393$, $p = 0.001$).

Spatiotemporally ubiquitous taxa plotted near the centre of species ordination (Figure 6.14), whilst the positioning of others (e.g. Simuliidae) could be linked to their temporal occurrence. Other taxa plotted according to their spatial distribution, for example Sphaeriidae occurred almost exclusively at site 1 and had a low axis 1 score despite their temporal occurrence. Accordingly, taxa such as *Oulimnius* larvae, which were largely found at sites 2 and 4, scored highly on axis 1 (Figure 6.14).

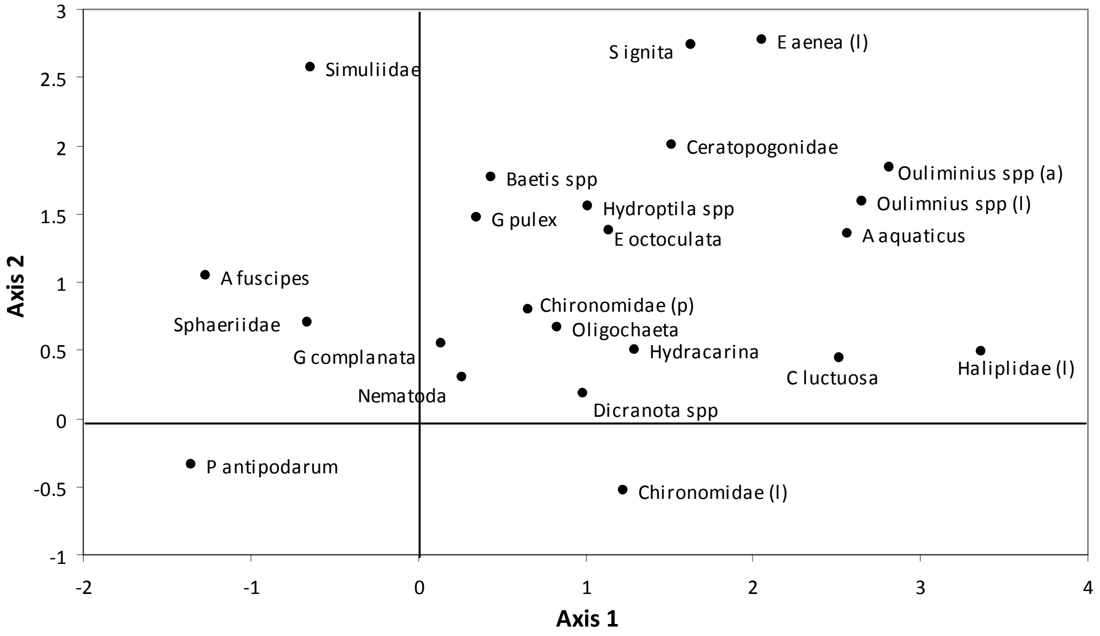


Figure 6.14: Detrended correspondence analysis species plot of benthic community data. All common invertebrate taxa (those accounting for >0.5% of the total community) are indicated.

6.5.2 Community metrics

Four metrics were calculated to summarise the benthic invertebrate community and allow characterisation of temporal change in community composition: total invertebrate abundance, taxon richness, the Berger-Parker dominance index and Simpson's Diversity Index (see section 4.9.1).

Total invertebrate abundance (TIA)

TIA varied between 84 individuals 0.1 m^{-2} at site 3 in June up to 2849 0.1 m^{-2} (mainly due to high chironomid densities) at site 4 in May. Spatial variation between sites was not significant (Table 6.9). Considering all sites, TIA was particularly high in May and lowest in August and September ($F_{4, 60} = 6.237$, $p < 0.001$; Table 6.8; Figure 6.15(i)). The interaction between TIA and site was significant ($F_{12, 48} = 4.195$, $p < 0.001$), and temporal change was only significant at site 4. Here abundance was highest in May and also high in July, then declined by 77 % between July and August and dropped further in September ($F_{1.456, 4.368} = 15.293$, $p = 0.012$).

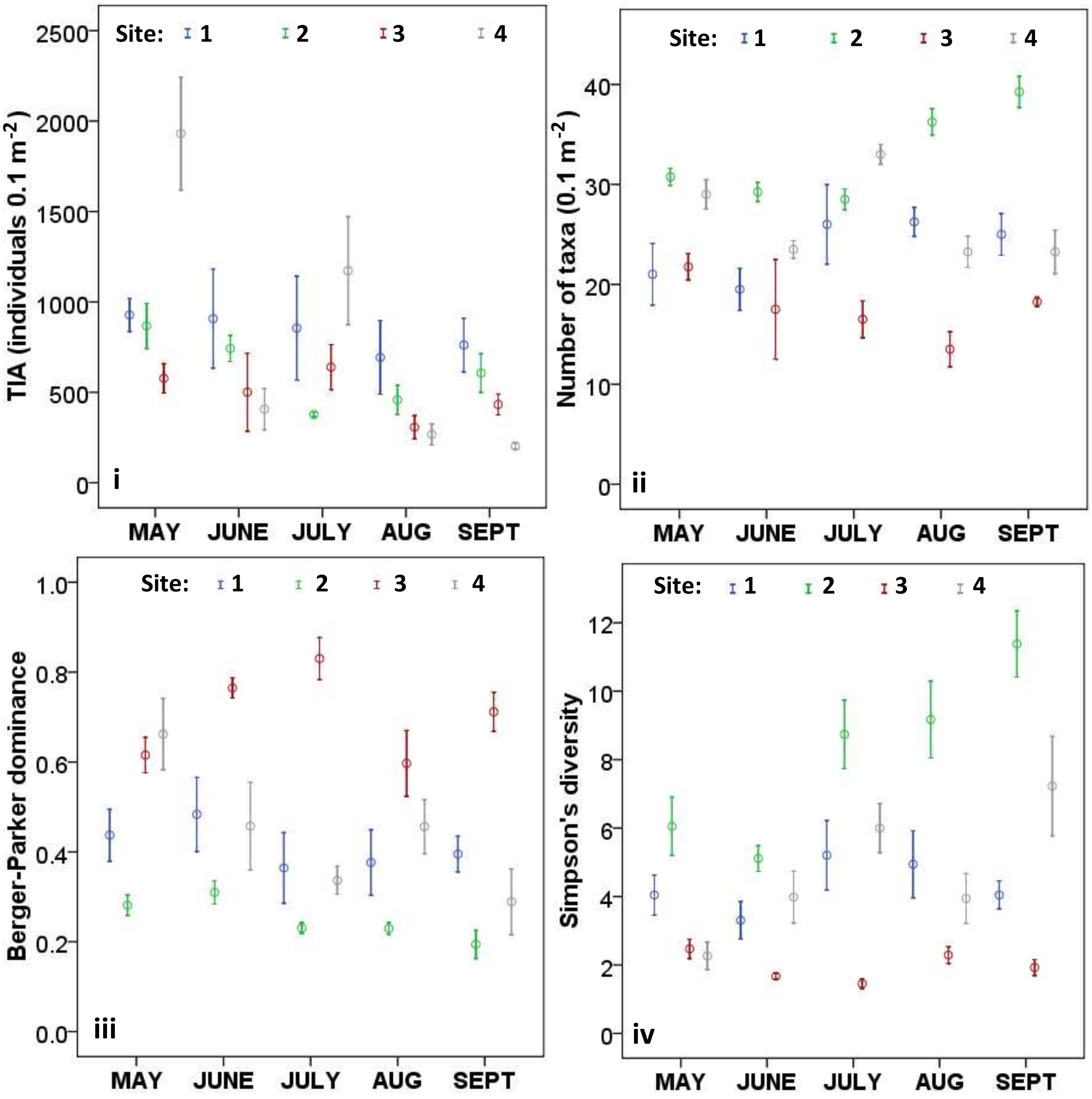


Figure 6.15: Mean ± 1 SE benthic community metrics: i) total abundance (TIA; individuals 0.1m^{-2}); ii) number of taxa (taxa 0.1m^{-2}); iii) Berger-Parker dominance; iv) Simpson's diversity.

Taxon richness

The number of taxa per sample (0.1 m^{-2}) varied between 5 taxa at site 3 in June to 42 taxa recorded at site 2 in September. Spatial differences in mean richness were site-specific, being highest at site 2 and particularly low at site 3 ($F_{3, 12}$ = 21.090, p <0.001; Table 6.9). Overall, mean richness was stable between months ($F_{4, 60}$ = 1.651, p = 0.173; Table 6.8; Figure 6.15(ii)); however the interaction between taxa and site was significant ($F_{12, 48}$ = 3.369, p = 0.001) and taxon richness varied significantly at two sites. At site 2, taxon richness was stable between May and July then increased to a September peak ($F_{2.188, 6.563}$ = 13.684, p = 0.004) due to increases in Gastropoda, Turbellaria and Coleoptera taxa. At site 4, the number of taxa was highest in July, with *Agabus didymus* and *Helophorus brevipalpis* (Coleoptera) occurring exclusively in this month; richness then declined by 29.5 % (10 taxa) in August and stayed low in September ($F_{1.774, 5.322}$ = 7.659, p = 0.029; Figure 6.15(ii)).

Berger-Parker dominance

Berger-Parker dominance varied between 0.15 in a site 4 September sample up to 0.94 in a chironomid-dominated site 3 sample taken in July. Spatial differences were site-specific, with dominance being particularly low at site 2, moderate at sites 1 and 4, and very high at site 3 ($F_{3, 12}$ = 57.793, p <0.001; Table 6.9). Overall, dominance was highest in May and June then declined in each subsequent month, but temporal change was not significant ($F_{2.186, 32.785}$ = 2.209, p = 0.122; Table 6.8; Figure 6.15(iii)). The interaction between dominance and site was, however, significant ($F_{12, 48}$ = 2.709, p = 0.007), with the overall temporal pattern being observed only at site 2 ($F_{2.017, 6.051}$ = 5.876, p = 0.038). Contrasting patterns were observed at other sites, for example dominance was stable at site 1, and was particularly variable at site 4, ranging from 0.66 ± 0.08 in May down to 0.29 ± 0.07 in September (Figure 6.15(iii)).

Table 6.8: Temporal change in benthic community metrics in the River Glen, May to September 2008

Community metric	**May**	**June**	**July**	**August**	**Sept**	**Temporal change**
Total invertebrate abundance (ind. 0.1m^{-2})	1075 ± 154	639 ± 98	761 ± 122	431 ± 68	500 ± 69	**
Number of taxa (taxa 0.1m^{-2})	25.6 ± 1.4	22.4 ± 1.7	26.0 ± 1.9	24.8 ± 2.2	26.4 ± 2.2	ns
Berger-Parker dominance	0.50 ± 0.05	0.50 ± 0.05	0.44 ± 0.06	0.41 ± 0.04	0.40 ± 0.05	ns
Simpson's diversity	3.7 ± 0.5	3.5 ± 0.4	5.3 ± 0.8	5.1 ± 0.8	6.1 ± 1.0	**

All values presented as mean ± 1 SE. Temporal change analysed using one-way RM ANOVA, with * and ** indicating overall significance levels of p <0.01 and n/s indicating p >0.05. Key – ind. = individuals.

Table 6.9: Spatial differences in benthic community metrics at River Glen sites 1-4

Community metric	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Spatial change**
Total invertebrate abundance (ind. 0.1m^{-2})	829 ± 87	610 ± 54	491 ± 56	796 ± 172	ns
Number of taxa (taxa 0.1m^{-2})	23.6	32.8	17.5	26.4	**
Berger-Parker dominance	0.41 ± 0.03	0.25 ± 0.01	0.70 ± 0.03	0.44 ± 0.04	**
Simpson's diversity	4.3 ± 0.34	8.1 ± 0.63	2.0 ± 0.12	4.7 ± 0.53	**

All values presented as mean ± 1 SE. Spatial change analysed using two-way RM ANOVA, with * and ** indicating overall significance levels of p <0.01 and n/s indicating p >0.05. Key – ind. = individuals.

Simpson's diversity

Diversity was very low (1.12) in the chironomid-dominated sample taken at site 3 in July, whilst the highest value (13.2) was recorded at site 2 in September. Spatial variability was the opposite of that reported for dominance, being very low at site 3 and particularly high at site 2 ($F_{3, 12} = 41.064$, $p < 0.001$; Table 6.9)). Temporally, diversity was lowest in May and June and highest in September; temporal change was significant ($F_{2.390, 35.850} = 6.386$, $p = 0.003$; Table 6.8; Figure 6.15(iv)), as was the interaction with site ($F_{6.511, 26.042} = 3.975$, $p = 0.005$). Temporal change was only significant at site 2, with diversity being low in June then increasing monthly to a September peak ($F_{1.616, 4.849} = 11.872$, $p = 0.015$; Figure 6.15(iv)).

6.5.3 Abundance of common taxa

Taxon abundances were examined firstly, to determine changes in the occurrence of dominant species with the potential to increase the strength of biotic interactions, and secondly, to consider changes in abundance resulting from variation in hydrological, environmental or biotic variables. Thirteen taxa each accounted for >1 % TIA and these taxa cumulatively comprised 88.7 % of the benthic community (Table 6.10). In addition, three regionally or nationally notable species were present at very low abundance: *Mesovelia furcata* (Hemiptera), the genus *Riolus* (Coleoptera) and *Gyrinus urinator* (Coleoptera) (Chadd and Extence, 2004).

Table 6.10: Occurrence of common benthic invertebrates (>1 % total invertebrate abundance) in the River Glen

Taxon	**Total no. of individuals**	**% of community**	**Cumulative % of community**	**Present in *x* % of samples**
CHIRONOMIDAE (l)	19532	35.8	35.8	100
OLIGOCHAETA	8637	15.8	51.7	100
Baetis spp.	4381	8.0	59.7	85
Potamopyrgus antipodarum	3878	7.1	66.8	40
SPHAERIIDAE	2935	5.4	72.1	81.3
Simuliidae (l)	1993	3.7	75.8	55
Oulimnius spp. (l)	1347	2.5	78.3	73.8
Gammarus pulex	1086	2.0	80.3	92.5
HYDRACARINA	1004	1.8	82.1	90
Erpobdella octoculata	986	1.8	83.9	97.5
Caenis luctuosa group	944	1.7	85.7	60
Hydroptila spp. (l)	875	1.6	87.2	63.8
Serratella ignita	769	1.4	88.7	31.3
Total	48367	88.7	88.7	

Chironomidae larvae

Larvae of the family Chironomidae (Diptera) experience seasonal changes in abundance due to adult emergence, but were by far the most abundant benthic taxon, accounting for 35.8 % TIA and occurring in all samples, and are therefore considered briefly here. Spatially, chironomids were more abundant at both East Glen sites compared with the West Glen ($F_{1, 14}$ = 46.517, p <0.001; Table 6.12). Temporally, chironomid abundance was highest in May and lowest in August and September ($F_{2.282, 34.232}$ = 9.008, p <0.001; Table 6.11); the interaction with site was significant ($F_{3, 12}$ = 16.568, p <0.001), but minor variations from the overall pattern of change were not significant at individual sites.

Table 6.11: Temporal change in the abundance of common benthic taxa in the River Glen, May to September 2008.

	Mean ± 1 SE abundance (individuals 0.1m^{-2})					Temporal change
	May	June	July	August	Sept	
CHIRONOMIDAE (l)	520 ± 154	232 ± 56	287 ± 66	94 ± 21	88 ± 34	**
OLIGOCHAETA	132 ± 23	125 ± 31	110 ± 26	89 ± 14	84 ± 19	ns
P. antipodarum	52 ± 33	65 ± 38	56 ± 34	23 ± 17	46 ± 34	ns
SPHAERIIDAE	18 ± 11	30 ± 17	33 ± 18	62 ± 44	39 ± 20	ns
SIMULIIDAE (l)	58 ± 35	13 ± 9	13 ± 6	20 ± 11	21 ± 17	ns
Gammarus pulex	9.6 ± 3.4	8.9 ± 2.0	24.8 ± 9.0	9.3 ± 2.9	15.3 ± 4.3	ns
HYDRACARINA	9.8 ± 2.1	5.8 ± 2.6	39 ± 24	2.9 ± 0.7	5.3 ± 1.6	ns
Erpobdella octoculata	15 ± 3.6	12 ± 2.7	8.4 ± 1.3	12 ± 2.2	14 ± 4.2	ns

All values presented as mean ± 1 SE. Temporal change analysed using one-way RM ANOVA; * indicates p <0.05, ** indicates p <0.01

Table 6.12: Spatial differences in the abundance of common benthic taxa at River Glen sites 1-4.

	Mean ± 1 SE abundance (individuals 0.1m^{-2})				Spatial change
	Site 1	Site 2	Site 3	Site 4	
CHIRONOMIDAE (l)	111 ± 30	112 ± 21	355 ± 51	398 ± 135	**
OLIGOCHAETA	133 ± 29	104 ± 18	69 ± 10	126 ± 21	ns
P. antipodarum	1189 ± 44	0	4.9 ± 1.2	0	*
SPHAERIIDAE	133 ± 36	6.8 ± 1.2	3.3 ± 0.8	3.1 ± 1.0	*
SIMULIIDAE (l)	81 ± 30	17 ± 8	0	0.9 ± 0.5	ns
Gammarus pulex	23.9 ± 5.0	14.0 ± 3.0	1.9 ± 0.4	14.6 ± 6.2	**
HYDRACARINA	6.1 ± 2.1	5.8 ± 1.3	4.1 ± 1.0	34.4 ± 19.5	**
Erpobdella octoculata	7.3 ± 1.4	26 ± 3.2	11.5 ± 1.5	4.6 ± 0.8	**

All values presented as mean ± 1 SE. Spatial change analysed using one-way RM ANOVA; * indicates p <0.05, ** indicates p <0.01, ns indicates p >0.05

Oligochaeta

Species of the Oligochaeta comprised 15.8 % TIA and were present in all samples at mean densities of 108 ± 11 0.1 m^{-2}, occurring at comparable abundance at all sites (Table 6.12). Oligochaete abundance peaked in May then declined in each subsequent month, but temporal change was not significant ($F_{4, 60} = 0.826$, $p = 0.514$; Table 6.11); neither was there a significant interaction with site.

Potamopyrgus antipodarum

P. antipodarum (Gastropoda) accounted for 7.1 % TIA but was patchily distributed, occurring in 40 % of samples at densities of ≤560 0.1 m^{-2}. *P. antipodarum* was more abundant at perennial than intermittent sites ($F_{1, 14} = 7.169$, $p = 0.018$), being particularly common at site 1 and absent from sites 2 and 4 (Table 6.12). Abundance was low in August and twice as high in other months but temporal change was not significant ($F_{1.758, 26.364} = 1.082$, $p = 0.346$; Table 6.11).

Sphaeriidae

Species of the family Sphaeriidae (Bivalvia) comprised 5.4 % TIA and were widespread, occurring in 81.3 % of samples at mean densities of 36 ± 11 0.1 m^{-2}, but being particularly abundant at site 1 ($F_{3, 12} = 7.736$, $p = 0.004$; Table 6.12). Abundances were lowest in May then increased gradually to a peak in August; however, neither temporal change nor interactions with spatial parameters were significant (Table 6.11).

Gammarus pulex

The amphipod *G. pulex* comprised 2 % TIA and was widespread, occurring in 92.5 % of samples at densities of ≤120 0.1 m^{-2}. Mean abundance of *G. pulex* was comparable at sites 1, 2 and 4 and low at site 3 ($F_{3, 12} = 8.504$, $p = 0.003$; Table 6.12). Considering all sites, *G. pulex* abundance was similar and low in May, June and August and highest in July, however, these changes were not significant ($F_{2.002, 30.027} = 2.354$, $p = 0.112$; Table 6.11). The interaction with site was significant ($F_{12, 48} = 2.811$, $p = 0.005$), with the overall pattern representing conditions only at site 1. At site 2, *G. pulex* abundance increased each month to a September peak, at site 3, abundance remained low in all months, and at site 4, abundance was 4x higher in July than in other months then fell sharply in August and remained low in September; temporal change was not significant at individual sites.

Hydracarina

The Hydracarina (Acari) accounted for 1.8 % of all invertebrates and was widespread, occurring in 90 % of samples at mean densities of 12.6 ± 5.0 0.1 m^{-2}. Abundance varied between sites, the taxon occurring at comparable low densities at sites 1-3 but being far more abundant at site 4 ($F_{3, 12} = 7.747$, $p = 0.004$; Table 6.12). Hydracarina were considerably more abundant in July than in any other month, but this temporal variability was not significant ($F_{1.518, 22.772} = 2.591$, $p = 0.108$; Table 6.11). The interaction between abundance and site was significant ($F_{5.747, 22.988} = 5.300$, $p = 0.002$), and the July peak occurred only at site 4, where it was followed by a reduction in abundance of >99 % in August ($F_{1.164, 1.448} = 6.937$, $p = 0.065$).

Erpobdella octoculata

E. octoculata (Hirudinea) accounted for 1.8 % TIA and was very widespread, occurring in 97.5 % of samples at densities of ≤60 0.1 m^{-2}. The taxon occurred at comparable densities at sites 1, 3 and 4, with significant spatial variability reflecting high abundances at site 2 ($F_{3, 12} = 12.278$, $p = 0.001$; Table 6.12). *E. octoculata* declined in abundance between May and July then became increasingly common in subsequent months, but temporal change was not significant ($F_{4, 60} = 1.007$, $p = 0.411$; Table 6.11). The interaction with site was significant ($F_{12, 48} = 4.553$, $p < 0.001$), with the overall pattern being observed and significant at site 2 ($F_{2.180, 6.539} = 7.940$, $p = 0.017$); contrasting patterns were recorded elsewhere, for example at site 4 abundance increased to a July peak then declined in both August and September, but temporal change was not significant at any other site.

Other common taxa

Baetis spp., Simuliidae, *Caenis luctuosa* group, *Hydroptila* spp., *Ouliminius* spp. and *Serratella ignita* were all common members of the benthic community (Table 6.10). However, larvae and pupae of the Insecta are commonly absent from the aquatic environment in summer following emergence of terrestrial adult life stages (Williams and Feltmate, 1992; Elliott, 2006); any attempt to relate their abundance to hydrological conditions is liable to be confounded by seasonal changes. They are therefore not appropriate for further consideration in the current investigation, except to note that Simuliidae larvae abundance declined considerably between May (58 ± 35 0.1 m^{-2}) and June (13 ± 9 0.1 m^{-2}; Table 6.11).

6.6 Spatiotemporal variability in the hyporheic invertebrate community

The composition of the community resident in the hyporheic zone is examined using the same methods as applied to benthic community data (aim 2, objective 1). Particular attention is paid to temporal change in the hyporheic occurrence of predominantly benthic taxa (objective 2).

Community description

A total of 5165 invertebrates were recorded in 232 samples pumped from the hyporheic zone. Of these, 2169 were from a depth of 10 cm, 1326 from 20 cm and 1670 from 30cm. At least 63 taxa were present, including 37 identified to species level and 26 higher taxa that probably contained multiple representatives.

6.6.1 Detrended correspondence analysis

Preliminary DCA indicated that axes scores (and therefore community composition) were comparable at the three hyporheic depths ($p > 0.5$) and all were therefore combined in the subsequent analyses. DCA was used to examine both spatial and temporal variability in community composition (Figure 6.16). Axis 1 explained 10.8 % of the species variation and was significantly correlated with several common taxa, in particular Simuliidae larvae and *Caenis luctuosa* group ($p < 0.001$). Axis 2, which explained an additional 9.8 % of the variation, had highly significant ($p < 0.001$) correlations with taxa including Simuliidae larvae and Sphaeriidae.

Temporal variability

Samples from all months formed an overlapping cluster towards the centre of the ordination (Figure 6.16(i)). May samples formed a relatively tight group that overlapped with all other months, whilst September samples spanned Axis 1 and June samples were particularly variable on axis 2. Despite this variability, temporal change was significant on both axes. Axis 1 scores declined between May and July then increased to a peak in September ($F_{2.802,\ 109.274} = 7.490$, $p < 0.001$), whilst axis 2

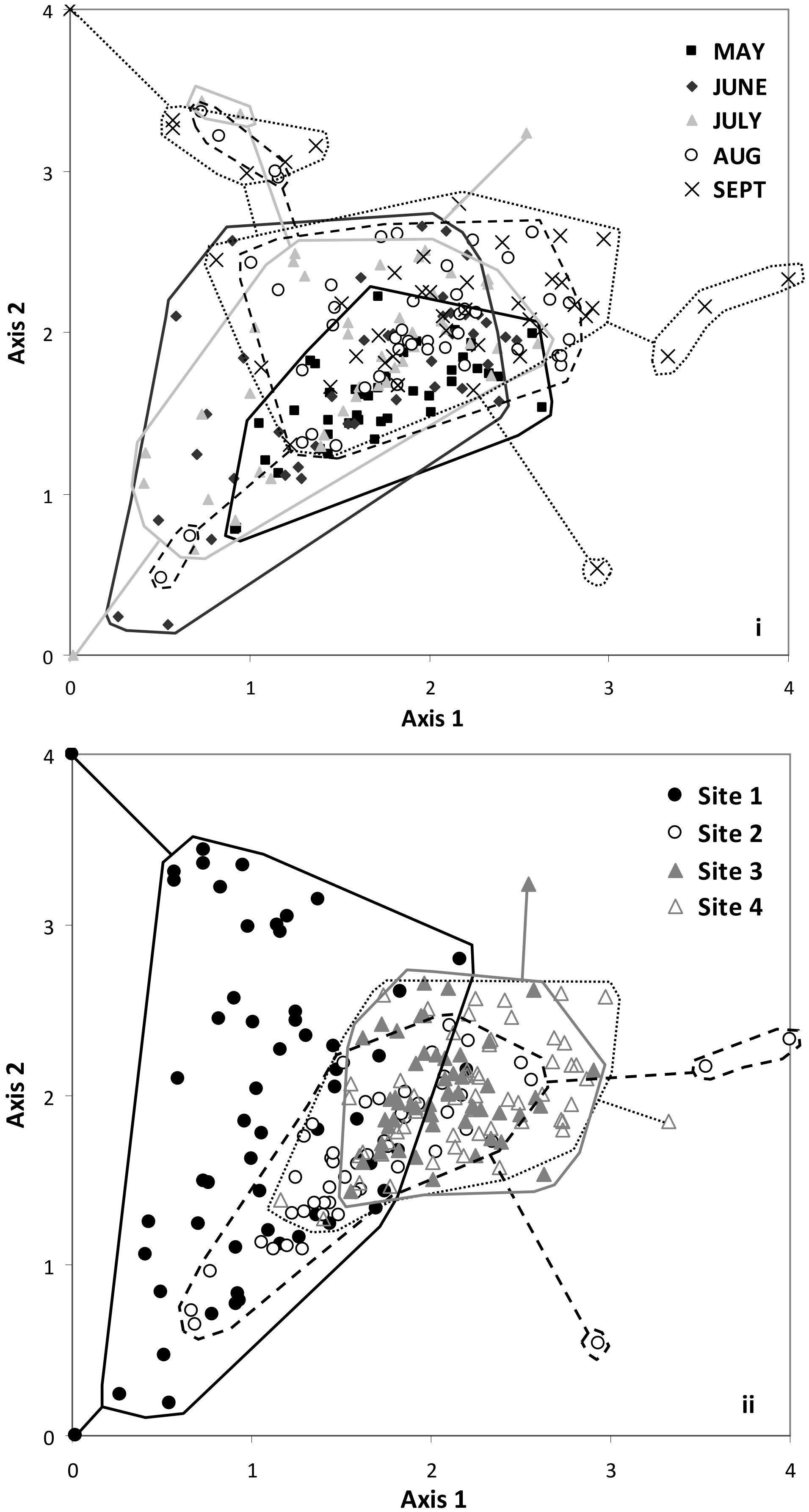


Figure 6.16: Detrended correspondence analysis sample plot of the hyporheic community: i) temporal variability; ii) spatial variability. Key: circles = West Glen; triangles = East Glen.

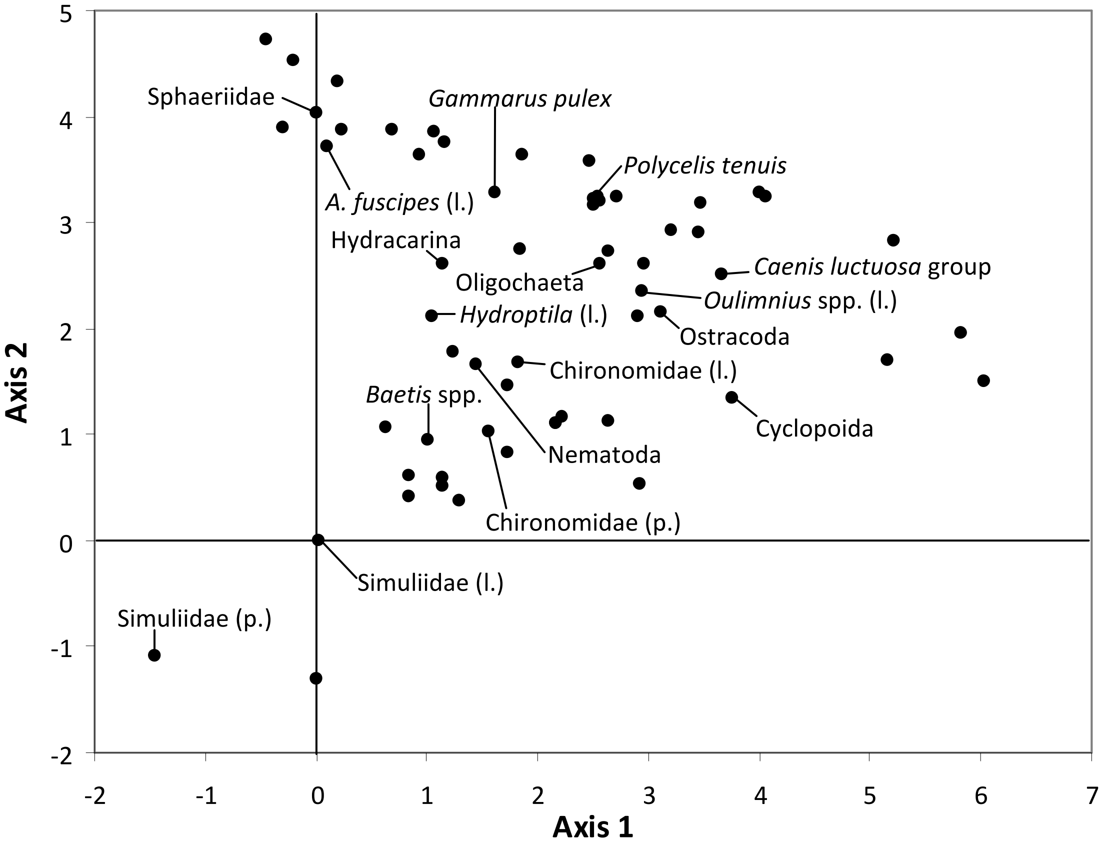


Figure 6.17: Detrended correspondence analysis species plot of hyporheic community data. All common invertebrate taxa (>0.5 % of the total community) are indicated.

scores were lowest in May then increased each month to a peak in September ($F_{2.997, 116.895} = 17.541$, $p < 0.001$; Figure 6.16(i)). Significant interactions between site and axis scores indicated that patterns of temporal change were site-specific.

Positioning of some taxa on the species plot (Figure 6.17) could be attributed to their temporal occurrence, for example the Sphaeriidae were most abundant in August and September and had high axis 2 scores. Similarly, abundance of Simuliidae larvae peaked in June and was very low in September, as reflected by low axis 2 scores. Ubiquitous taxa such as the Chironomidae plotted towards the centre of the species plot despite significant temporal changes in their abundance.

Spatial variability

Whilst the tight clustering of samples into site-specific groups observed for the benthic community was absent, hyporheic communities of certain sites could be distinguished (Figure 6.16(ii)). In particular, site 1 samples spanned the length of axis

2, indicating community variability, but had low axis 1 scores. In contrast, site 3 and 4 samples formed a relatively tight cluster at the centre of the ordination, indicating community homogeneity; these samples had higher axis 1 scores than West Glen sites. Between-site variability was significant on axis 1 ($F_{3, 36}$ = 100.105, p <0.001) whilst axis 2 scores were comparable at all sites (Figure 6.16(ii)).

The association of certain taxa with specific sites was reflected by their positioning in the species plot (Figure 6.17). *Caenis luctuosa* group, for example, occurred at all sites except site 1 and scored highly on axis 1, whilst most Simuliidae larvae were recorded at site 1 and had a much lower axis 1 score.

6.6.2 Community metrics

Total invertebrate abundance (TIA)

TIA peaked at 136 6 L^{-1} in a 10 cm depth sample taken at site 4 in May, whilst no invertebrates were present in six samples. Abundance was comparable at 10 cm (27 ± 3.3 6 L^{-1}) and 30 cm (23 ± 3.2 6 L^{-1}) but lower at 20 cm (16 ± 2.4 6 L^{-1}; $F_{2, 41}$ = 5.158, p = 0.010). The interaction with hyporheic depth was not significant ($F_{8, 164}$ = 0.585, p = 0.790) and all depths were therefore pooled in subsequent analyses. Spatially, TIA was comparable at sites 1, 2 and 3 and higher at site 4 ($F_{3, 40}$ = 8.224, p <0.001; Table 6.14). TIA was highest in May, moderate between June and August and lowest in September ($F_{4, 172}$ = 9.927, p <0.001; Table 6.13; Figure 6.18(i)). The interaction between TIA and site was significant ($F_{12, 160}$ = 3.167, p <0.001) and patterns of temporal change were site-specific. At site 1, TIA was highest in June but temporal change was not significant; at site 2, the overall pattern was observed ($F_{4, 28}$ = 6.224, p = 0.001); the same pattern was seen at site 3 but was not significant; and at site 4, there was a pronounced peak in abundance in May (75 ± 12 6 L^{-1}) and abundance was also high in July ($F_{4, 44}$ = 10.864, p <0.001).

Taxon richness

Taxon richness peaked at 16 taxa 6 L^{-1} in a sample taken at site 2 in August, whilst no invertebrates were recorded in six samples. The number of taxa was higher at 10 cm (6.0 ± 0.3 taxa 6 L^{-1}) than at both 20 cm (3.9 ± 0.3 taxa 6 L^{-1}) and 30 cm (4.3 ± 0.3 taxa 6 L^{-1}; $F_{2, 41}$ = 7.609, p = 0.002), but the interaction with depth was not significant ($F_{8, 164}$ = 0.937, p = 0.487). Taxon richness was particularly low at site 3 and was highest at site 4 ($F_{3, 40}$ = 4.604, p = 0.007; Table 6.14). Temporally, mean taxon richness was high in May, moderate from June to August and low in September ($F_{4, 172}$ = 3.016, p = 0.024; Table 6.13; Figure 6.18(ii)), and the interaction between taxa and site was not significant ($F_{12, 160}$ = 1.384, p = 0.178).

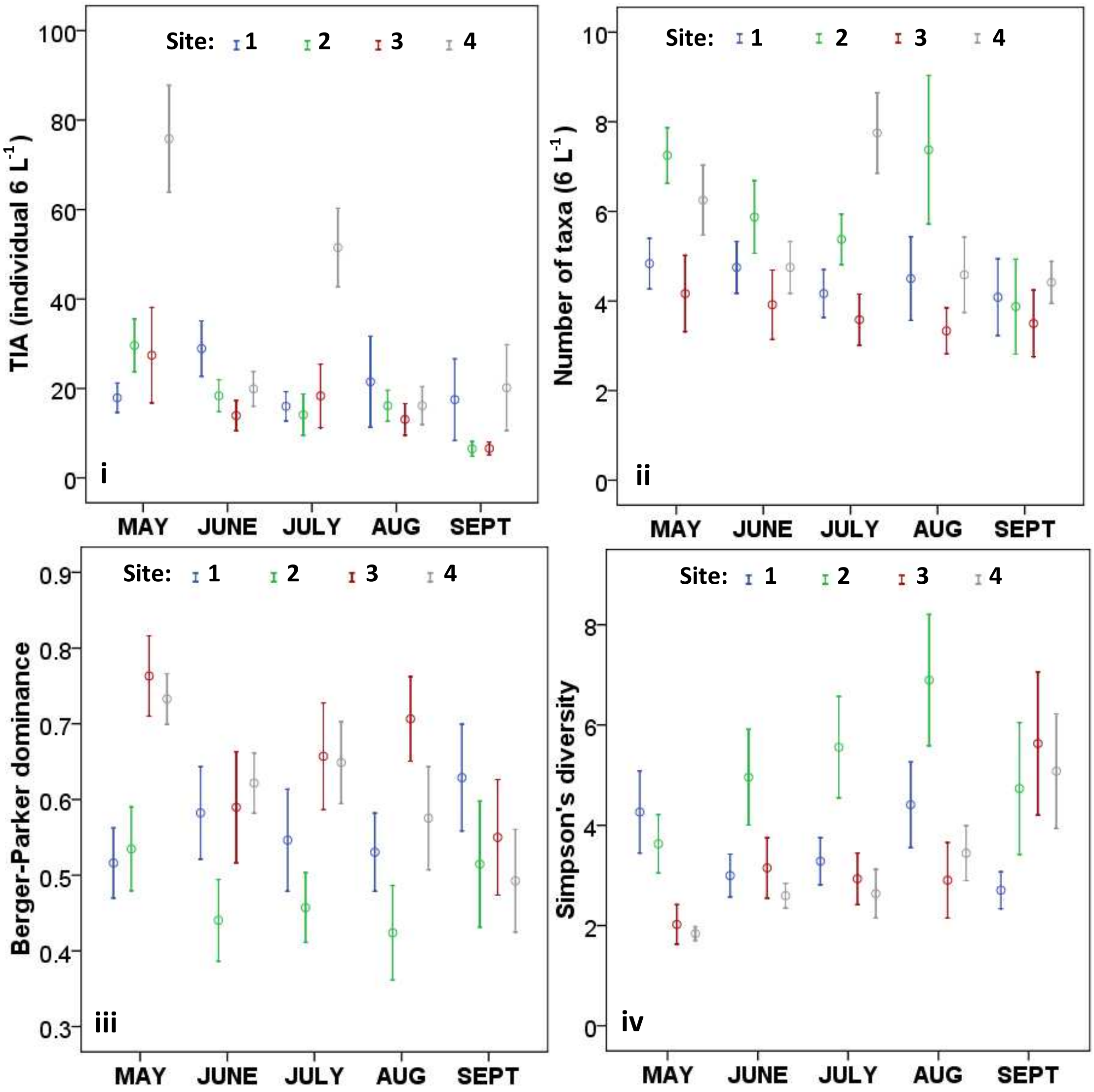


Figure 6.18: Mean ± 1 SE temporal change in hyporheic community metrics: i) total invertebrate abundance (TIA; individuals 6 L^{-1}); ii) number of taxa (taxa 6 L^{-1}); iii) Berger-Parker dominance; iv) Simpson's diversity

Table 6.13: Temporal change in hyporheic community metrics in the River Glen, May to September 2008

Community metric	May	June	July	August	Sept	Temporal change
Total abundance (individuals 6 L^{-1})	38.4 ± 5.6	20.5 ± 2.4	26.0 ± 4.0	16.8 ± 3.1	13.3 ± 3.7	**
Taxon richness (taxa 6 L^{-1})	5.5 ± 0.4	4.7 ± 0.3	5.2 ± 0.4	4.7 ± 0.5	4.0 ± 0.4	*
Berger-Parker dominance	0.64 ± 0.03	0.58 ± 0.03	0.58 ± 0.03	0.58 ± 0.03	0.55 ± 0.04	ns
Simpson's diversity	2.9 ± 0.3	3.3 ± 0.3	3.6 ± 0.4	4.1 ± 0.5	4.7 ± 0.6	*

Values presented as mean ± 1SE of all samples. Temporal change analysed using one-way RM ANOVA; * indicates p <0.05, ** indicates p <0.01, ns indicates p >0.05.

Table 6.14: Spatial differences in hyporheic community metrics at River Glen sites 1-4

Community metric	Site 1	Site 2	Site 3	Site 4	Spatial change
Total abundance (individuals 6 L^{-1})	20.4 ± 3.1	15.2 ± 1.7	15.9 ± 2.8	36.7 ± 4.7	**
Taxon richness (taxa 6 L^{-1})	4.5 ± 0.3	5.4 ± 0.4	3.7 ± 0.3	5.6 ± 0.4	**
Berger-Parker dominance	0.56 ± 0.03	0.47 ± 0.03	0.66 ± 0.03	0.61 ± 0.03	**
Simpson's diversity	3.5 ± 0.3	5.3 ± 0.5	3.3 ± 0.4	3.1 ± 0.3	**

Values presented as mean ± 1SE of all samples. Spatial change analysed using two-way RM ANOVA; ** indicates p <0.01

Berger-Parker dominance

Dominance ranged from 1 (complete dominance) in several samples containing a single taxon, down to 0.14 in a sample taken from site 2 in September, which contained seven taxa at low abundance. Dominance values were relatively low at a depth of 10 cm (0.52 ± 0.02) and higher at 20 cm (0.61 ± 0.03) and 30 cm (0.60 ± 0.02; $F_{2, 40} = 4.460$, $p = 0.018$). Site-specific differences were significant ($F_{3, 39} = 7.049$, $p = 0.001$), with mean dominance being lowest at site 2 and highest at site 3 (Table 6.14). Dominance was highest in May, stable and moderate between June and August, and lower in September (Table 6.13; Figure 6.18(iii)); temporal change was not significant.

Simpson's diversity

The lowest diversity value possible (1) was recorded in all samples containing a single taxon, whilst diversity peaked at 16.5 in a site 3 sample taken in September; this sample comprised nine taxa at low abundance. Diversity peaked at 10 cm but was comparable at all hyporheic depths ($F_{2, 40} = 2.117$, $p = 0.134$). Spatially, diversity was comparable at sites 1, 3 and 4 and significantly higher at site 2 ($F_{3, 39}$ = 8.075, p <0.001; Table 6.14). Overall, diversity increased gradually from May to a peak in September ($F_{2.828, 118.774}$ = 2.776, p = 0.047; Figure 6.18(iv); Table 6.14). However, the interaction between site and diversity was significant ($F_{8.505, 110.569}$ = 2.009, p = 0.048), with the overall pattern being observed at sites 3 and 4; temporal change was not significant at sites 1 or 2 (Figure 6.18(iv)).

6.6.3 Abundance of common taxa

The abundance of taxa common in the hyporheic zone was examined, primarily to identify any changes in the use of the hyporheic zone by benthic invertebrates. Eleven taxa each comprised >1 % TIA, and together these taxa accounted for 90.8 % of the hyporheos (Table 6.15). In addition to these common taxa, two regionally-notable species of conservation interest were present at very low abundance: *Mesovelia furcata* (also present in the benthic sediments) and the groundwater specialist *Niphargus aquilex* (Amphipoda) (Chadd and Extence, 2004).

Table 6.15: Occurrence of common invertebrates (>1 % total invertebrate abundance) in the hyporheic zone of the River Glen

Taxon	Total no. of individuals	% of community	Cumulative % of community	Present in *x* % of samples
CHIRONOMIDAE (l)	2133	41.3	41.3	85.3
OLIGOCHAETA	849	16.4	57.7	60.0
SIMULIIDAE (l)	440	8.5	66.3	31.5
SPHAERIIDAE	323	6.3	72.5	19.0
Baetis spp.	268	5.2	77.7	33.6
NEMATODA (meiofauna)	186	3.6	81.3	31.9
CYCLOPOIDA (meiofauna)	140	2.7	84.0	21.6
CERATOPOGONIDAE (pupae)	122	2.4	86.4	1.7
HYDRACARINA	82	1.6	88.0	21.1
Caenis luctuosa group	79	1.5	89.4	18.9
Gammarus pulex	67	1.3	90.8	14.7
Total	4689	90.8		

Chironomidae larvae

Chironomid larvae dominated the hyporheic community, accounting for 41.3 % TIA and occurring in 85.3 % of samples at mean densities of 9.2 ± 1.1 6 L^{-1}. Vertically, the taxon was similarly abundant at depths of 10 cm and 30 cm, but less common at 20 cm ($F_{2, 41}$ = 4.938, p = 0.012). Spatially, chironomids were less numerous at West Glen than East Glen sites ($F_{1, 42}$ = 17.695, p <0.001), with particularly high abundance recorded at site 4 (Table 6.17). Chironomid abundance was particularly high in May, declined sharply in June and continued to fall in each subsequent month ($F_{2.469, 106.159} = 30.402$, $p < 0.001$; Table 6.16); the interaction with site was significant ($F_{9.277, 123.688} = 4.734$, $p < 0.001$). At site 1, May abundances were only slightly higher than in subsequent months; at sites 2 and 3, abundance rose between July and August before falling to a five-month low in September; and at site 4, chironomid abundance was particularly high in May and very low in August and September; temporal change was significant at all individual sites ($p \leq 0.047$).

Oligochaeta

Species of oligochaete comprised 16.4 % TIA and occurred in 60 % of samples at mean densities of 3.7 ± 0.8 6 L^{-1}; the taxon declined in abundance with increasing depth but this was not significant. Oligochaetes were more common on the East Glen than the West Glen ($F_{1, 42} = 4.338$, $p = 0.043$), with numbers being highest at site 4 (Table 6.17). Overall, oligochaete abundance was lowest in May and June then peaked in July, but temporal change was not significant ($F_{2.321, 99.797} = 1.802$, $p = 0.164$; Table 6.16); neither were there significant interactions with spatial parameters.

Simuliidae larvae, *Baetis* spp., Ceratopogonidae pupae and *Caenis luctuosa* group

These insect taxa were all common in the hyporheic zone, however, due to the confounding factor of seasonal adult emergence, patterns of change are not considered in detail. It is of interest to note, however, that Simuliidae larvae were particularly abundant in the hyporheic zone at sites 1 and 2 in June ($F_{1.764, 75.838} = 5.031$, $p = 0.012$; Tables 6.16 and 6.17).

Sphaeriidae

The family Sphaeriidae (Mollusca: Bivalvia) comprised 6.3 % of the hyporheos and were patchily distributed, being present in 19 % of samples at densities of ≤96 6 L^{-1}; densities were comparable at all hyporheic depths. Spatial variation in abundance was significant, with sphaeriids occurring at mean densities of 5.1 ± 1.8 6 L^{-1} at site 1 and being virtually absent from all other sites ($F_{3, 40} = 10.805$, $p < 0.001$; Table 6.17).

Considering all sites, sphaeriid abundance was lowest in May and peaked in August but temporal change was not significant overall ($F_{2.340,\ 100.602} = 2.166$, $p = 0.111$; Table 6.16) or at site 1.

Hydracarina

The Hydracarina accounted for 1.6 % TIA, equating to 82 individuals. The taxon occurred in 21.1 % of samples at densities of ≤5 6 L^{-1}; abundances were comparably low at all hyporheic depths, all sites and all months (Tables 6.16 and 6.17).

Table 6.16: Temporal change in the abundance of common taxa in the hyporheic zone of the River Glen, May to September 2008.

	Mean ± 1 SE abundance (individuals 6 L^{-1})					**Temporal change***
	May	**June**	**July**	**August**	**Sept**	
CHIRONOMIDAE (l)	25.9 ± 4.4	8.5 ± 1.1	7.2 ± 1.4	4.1 ± 0.9	1.6 ± 0.2	**
OLIGOCHAETA	2.0 ± 0.6	1.9 ± 0.5	5.7 ± 2.7	3.4 ± 0.8	5.0 ± 2.6	ns
SIMULIIDAE (l)	2.3 ± 0.7	4.5 ± 1.6	2.3 ± 0.8	0.6 ± 0.3	0.06 ± 0.04	*
SPHAERIIDAE	0.1 ± 0.07	1.0 ± 0.5	0.8 ± 0.3	3.1 ± 2.1	1.8 ± 1.0	ns
HYDRACARINA	0.2 ± 0.09	0.3 ± 0.1	0.5 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	ns
Gammarus pulex	0.2 ± 0.08	0.3 ± 0.07	0.8 ± 0.4	0.1 ± 0.07	0.08 ± 0.04	ns

*Analysis of data from all sites using one-way RM ANOVA; * indicates *p* ≤0.05, ** indicates *p* ≤0.01, ns indicates *p* >0.05

Table 6.17: Spatial differences in the abundance of common taxa in the hyporheic zone of the River Glen sites 1-4.

	Mean ± 1 SE abundance (individuals 6 L^{-1})				**Spatial change**
	Site 1	**Site 2**	**Site 3**	**Site 4**	
CHIRONOMIDAE (l)	4.3 ± 0.6	5.0 ± 1.0	9.5 ± 2.0	17.4 ± 3.3	**
OLIGOCHAETA	1.7 ± 0.6	1.7 ± 0.3	2.9 ± 1.4	8.0 ± 2.6	*
SIMULIIDAE (l)	5.1 ± 1.2	1.7 ± 0.4	0	1.8 ± 0.4	**
SPHAERIIDAE	5.1 ± 1.8	0.04 ± 0.03	0.2 ± 0.09	0.08 ± 0.04	**
HYDRACARINA	0.53 ± 0.14	0.35 ± 0.10	0.13 ± 0.06	0.40 ± 0.11	ns
Gammarus pulex	0.22 ± 0.06	0.08 ± 0.04	0.07 ± 0.03	0.08 ± 0.36	**

*Analysis of data from all sampling dates using two-way RM ANOVA; * indicates *p* ≤0.05, ** indicates *p* ≤ 0.01, ns indicates *p* >0.05

Gammarus pulex

The shrimp *Gammarus pulex* comprised 1.3 % of the hyporheos, with just 67 individuals being recorded from 14.7 % of samples. Densities peaked at 20 6 L^{-1}, however most samples contained only single specimens. Mean densities peaked at 10 cm then declined moderately with increasing depth. Inter-site variability was significant, with more individuals occurring at site 4 than at sites 1-3 combined ($F_{3,\ 40} = 6.694$, $p = 0.001$; Table 6.17). Overall, *G. pulex* abundance was threefold higher in July than in other months, but temporal change was not significant ($F_{2.395,\ 102.988}$ =

1.502, $p = 0.225$; Table 6.16). The interaction with site was, however, significant ($F_{8.065,\ 107.532} = 3.028$, $p = 0.004$), and the July peak resulted in significant temporal change at site 4 ($F_{1.615,\ 17.761} = 4.155$, $p = 0.040$).

6.7 Benthic invertebrate use of the hyporheic zone

To determine the extent to which the hyporheic zone habitat was used by benthic invertebrates, the hyporheic proportion of the total (benthic + hyporheic) community was calculated for each month (see Section 4.9.6; aim 2, objective 3). Four predominantly benthic non-insect macroinvertebrate taxa were selected for this analysis: Oligochaeta, Sphaeriidae, Hydracarina and *Gammarus pulex*. *G. pulex* abundance was low (a total of 67 individuals) but was included to allow comparison with the River Lathkill. Chironomidae larvae were also included due to their dominance in benthic and hyporheic communities, as were Simuliidae, due to preliminary observation of interesting patterns; these results should be treated with caution due to the complicating influence of seasonal adult emergence.

Total invertebrate abundance

The hyporheic proportion of TIA varied between sites, being comparable at sites 1, 2 and 3 (0.7-0.11 ± 0.01-0.02) and higher at site 4 (0.16 ± 0.02; $F_{3,\ 12} = 5.320$, $p = 0.015$). Overall, the hyporheic proportion of TIA was lowest in September and peaked in June but differences between months were not significant ($F_{4,\ 44} = 0.446$, $p = 0.775$), and nor was the interaction with site (Table 6.18).

Chironomidae larvae

The hyporheic proportion of the Chironomidae population was lowest at site 2 (0.1 ± 0.02) and was particularly high at site 4 (0.2 ± 0.03); spatial variation was not significant ($F_{2,\ 9} = 2.887$, $p = 0.108$). Considering all sites, the hyporheic proportion varied little between months (Table 6.18). Greater variation was observed at site 4, the proportion increasing between May (0.13 ± 0.02) and July (0.23 ± 0.12) then remaining high in subsequent months; however, temporal variation was not significant at this site or overall.

Table 6.18: The hyporheic proportion of the total (benthic + hyporheic) invertebrate community present on the River Glen, May to September 2008.

	May	June	July	August	Sept	Temporal change
TIA	0.10 ± 0.02	0.14 ± 0.03	0.011 ± 0.02	0.12 ± 0.02	0.09 ± 0.03	ns
Chironomidae	0.16 ± 0.03	0.15 ± 0.03	0.12 ± 0.03	0.16 ± 0.03	0.13 ± 0.03	ns
Gammarus pulex	0.05 ± 0.03	0.17 ± 0.09	0.04 ± 0.02	0.09 ± 0.06	0.09 ± 0.06	ns
Hydracarina	0.02 ± 0.01	0.14 ± 0.04	0.11 ± 0.04	0.26 ± 0.08	0.19 ± 0.05	ns
Oligochaeta	0.07 ± 0.03	0.10 ± 0.03	0.13 ± 0.04	0.10 ± 0.02	0.13 ± 0.06	ns
Simuliidae*	0.25 ± 0.12	0.60 ± 0.09	0.35 ± 0.07	0.07 ± 0.04	0.01 ± 0.01	**
Sphaeriidae	0.01 ± 0.01	0.15 ± 0.09	0.16 ± 0.07	0.11 ± 0.07	0.07 ± 0.03	ns

*West Glen sites (1 and 2) only

Oligochaeta

The hyporheic proportion of the oligochaete population varied from a low of 0.05 ± 0.02 at site 2 up to 0.18 ± 0.05 at site 4, but spatial variability was not significant. Temporally, the proportion of oligochaetes within the hyporheic zone was lowest in May and highest in July and September, but temporal changes were not significant ($F_{2.709, 26.797} = 0.682$, $p = 0.556$; Table 6.18).

Sphaeriidae

Sphaeriids were virtually absent from the hyporheic zone of site 2, whilst the hyporheic proportion of this taxon peaked at 0.17 ± 0.07 at site 3; spatial variability was not significant ($F_{3, 12} = 2.008$, $p = 0.167$). Temporally, the hyporheic proportion of the sphaeriid community was particularly low in May and considerably higher in June and July, but differences between months were not significant overall ($F_{2.294, 25.239} = 1.154$, $p = 0.337$; Table 6.18), or at site 1 (where the taxon was particularly abundant; Tables 6.12 and 6.17).

Hydracarina

The proportion of the total Hydracarina population inhabiting the hyporheic sediments varied between sites ($F_{3, 12} = 5.494$, $p = 0.013$), being comparable at sites 2-4 (0.9-0.12 ± 0.04) and higher at site 1 (0.27 ± 0.06). Temporally, the proportion of the Hydracarina population resident within the hyporheic zone was particularly low in May and more than tenfold higher in August, but differences between months were not significant ($F_{4, 44} = 2.159$, $p = 0.089$; Table 6.18).

Gammarus pulex

The hyporheic proportion of the *G. pulex* population varied between sites, being particularly high at site 4 (0.15 ± 0.07; $F_{3, 12} = 3.639$, $p = 0.045$). Overall, the hyporheic proportion was twice as high in June as in any other month and was lowest in July, although patterns of temporal change were site specific; at site 4, for example, the proportion increased each month to a August-September peak. However, abundances were low throughout and no temporal changes were significant (Table 6.18).

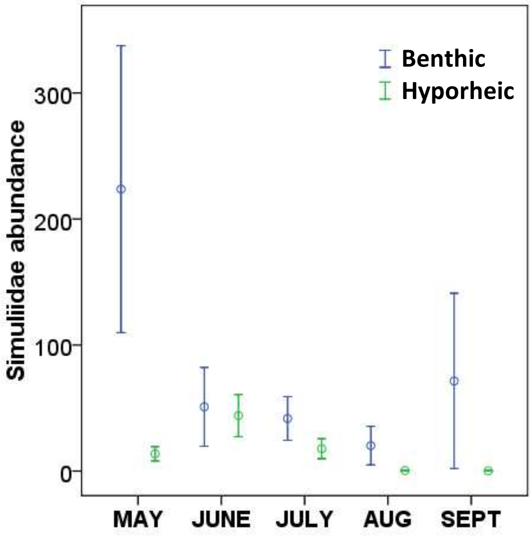


Figure 6.19: Mean ± 1 SE Simuliidae abundance in the benthic (0.1 m^{-2}) and hyporheic (6 L^{-1}) sediments, from which temporal change in hyporheic proportion can be inferred.

Simuliidae

Simuliidae larvae were largely restricted to the West Glen so only data from sites 1 and 2 were analysed. At these sites, the hyporheic proportion of the community was considerably higher in June than in any other month (the only taxon for which hyporheic exceeded benthic proportion in any month; Table 6.18), reflecting both decreased benthic abundance and increased hyporheic abundance (Figure 6.19); this was the only common taxa for which temporal change in the hyporheic proportion was significant ($F_{4, 28} = 9.618$, p <0.001; see also Figure 7.4).

6.8 Invertebrate-environment relationships

Canonical correspondence analysis (CCA) is used to examine relationships between invertebrate communities and environmental parameters, in both the benthic and the hyporheic zones (aim 2, objective 4). Pearson correlation coefficients were also calculated to assess relationships between environmental variables and abundance of common taxa in both benthic and hyporheic sediments, but this analysis yielded little useful information and is not presented.

6.8.1 Canonical correspondence analysis: benthic community

Sufficient data were available for the inclusion of three hydrological variables (depth, width, velocity) and five water chemistry variables (temperature, pH, conductivity, DO (concentration and % saturation)). Preliminary analyses were conducted using forward selection procedures with 999 iterations of the Monte Carlo random permutation test, to identify autocorrelated and non-significant variables. Performance of the model was enhanced by excluding DO (concentration) and retaining the remaining seven variables; all included variables then made a significant contribution to the explanatory power of the model ($p \leq 0.006$) and redundancy in the dataset was minimised (variance inflation factors ≤1.99). No arch effect was observed and detrending was therefore not necessary.

Spatial variability

Monte-Carlo tests indicated a significant contribution of both the first axis and the trace to model performance ($p = 0.001$) although the F-ratio was higher for axis 1 ($F = 10.311$) than for the trace ($F = 5.772$). Eigenvalues were low, suggesting that the environmental gradients influencing the community were weak (Table 6.19).

Axis 1 explained 12.5 % of the variance and was primarily correlated with depth and width, as well as pH (Table 6.19). Samples plotted according to site-specific differences in these variables, with little overlap between deep, low-pH perennial site 1 and shallow, high-pH intermittent sites 2 and 4 (Figure 6.20(i)). Differences in site axis scores were highly significant ($F_{3, 76} = 58.481$, $p < 0.001$). Whilst many abundant taxa were ubiquitous and plotted at the centre of the species plot, those with individual site associations were positioned accordingly, for example *P. antipodarum* was most abundant at site 1 and absent from sites 2 and 4, and scored highly on axis 1 (Figure 6.20(ii)). The second axis explained 9.8 % of the species data variation, was also most strongly correlated with depth and width. This axis primarily reflected tributary-specific hydrological variability, with deeper, faster-flowing West Glen sites having lower scores (Figure 6.20(i)). Other relevant variables included temperature, with particularly high temperatures at site 4 reflected by positioning of these samples exclusively in the positive quadrants of axis 2. The majority of common taxa plotted near the centre of axis 2, although *S. ignita* was largely restricted to site 2 and had a low axis score (Figure 6.20(ii)).

Temporal variability

CCA was repeated with site as a covariable to examine general patterns of temporal change (Figure 6.21; Table 6.19). Monte Carlo tests indicated that explanatory power of variables associated with both the first axis and the trace was significant (p = 0.001), although F –ratios were low for both axis 1 (F = 5.675) and the trace (F = 3.114). All eigenvalues were low, indicating that environmental gradients related to combinations of these variables were weaker than those related to site-specific variability (Table 6.19).

Axis 1 explained 6.9 % of the variance in the species data and had highly significant correlations with velocity and temperature. Axis 1 scores decreased between May and June and fell again in July then remained similar and low for the remainder of the study period ($F_{4, 75}$ = 29.243, p <0.001; Figure 6.21(i)); these changes reflected high temperatures in July and August and lower flow velocities from July onwards. All common taxa (>1 % TIA) plotted towards the centre of axis 1, indicating their occurrence throughout the range of environmental conditions, whilst taxa at the axis extremities represented very few (≤2) individuals (Figure 6.21(ii)). Axis 2 explained a further 4.9 % of the species data variance, and was significantly correlated with temperature and conductivity. Axis 2 scores were moderate in May and June, peaked in July then declined to a five-month low in September ($F_{4, 75}$ = 7.562, p <0.001). Again, changes were partly due to peak temperatures in July and August,

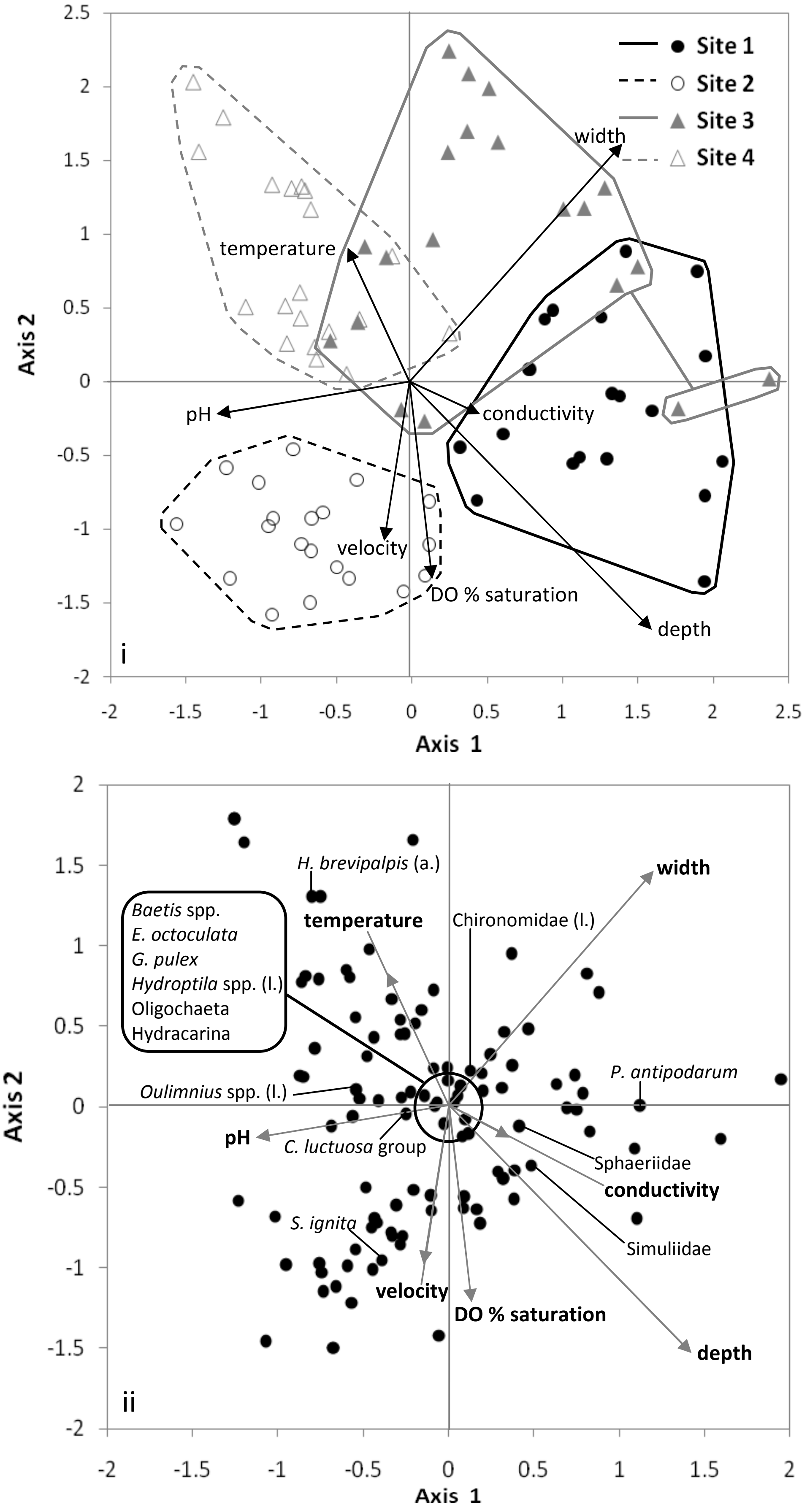


Figure 6.20: Canonical correspondence analysis ordination of benthic invertebrate and surface water data. Spatial variability: i) sample-environment biplot. Key: circles = West Glen; triangles = East Glen; ii) species-environment biplot.

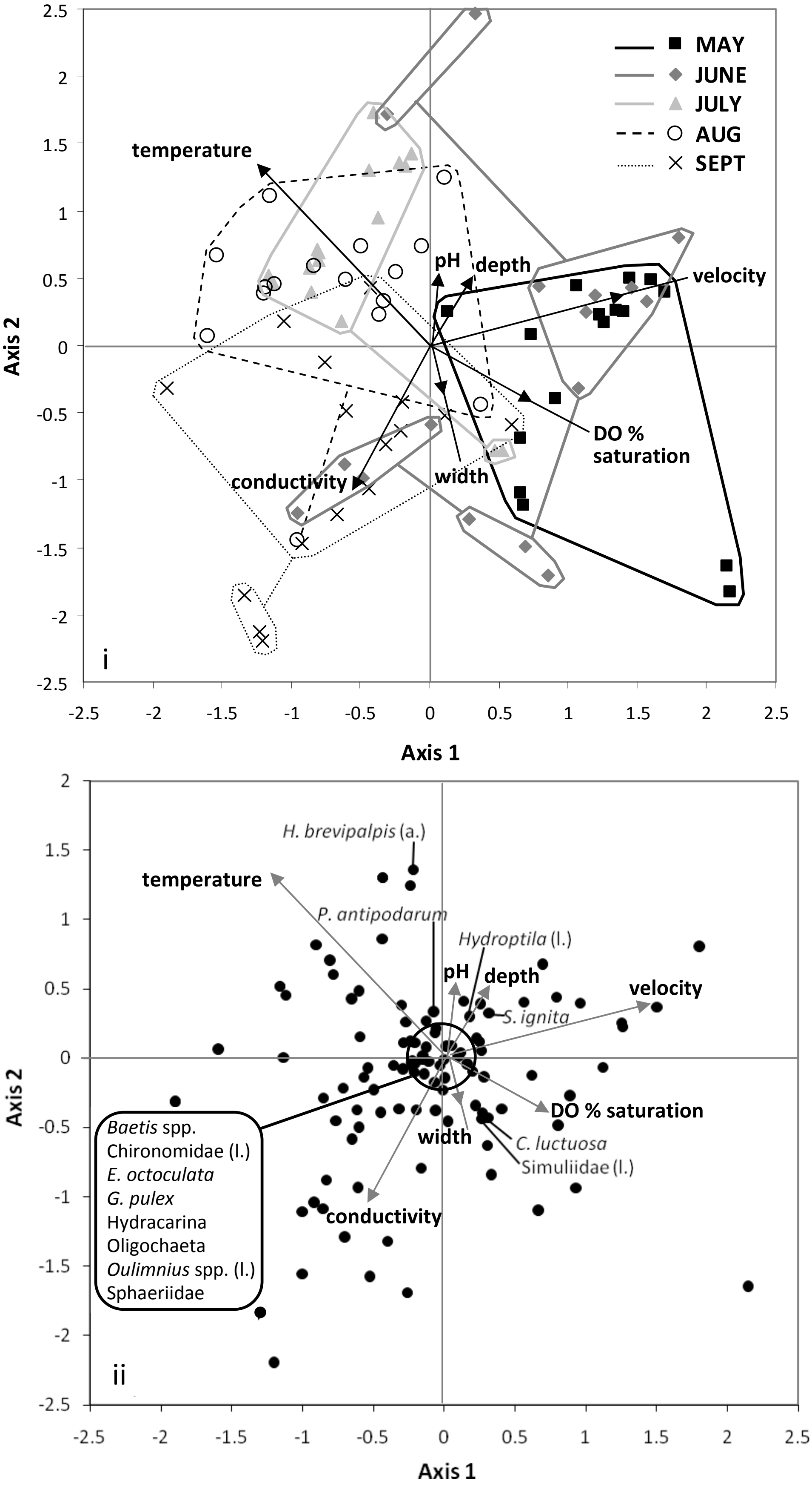


Figure 6.21: Canonical correspondence analysis ordination of benthic invertebrate and surface water data, with site as a covariable. Temporal variability: i) sample-environment biplot; ii) species-environment biplot.

and also reflected high conductivity in September. All common taxa plotted near the centre of axis 2, whilst semi-aquatic taxa (e.g. *Helophorus brevipalpis*) had high axis scores, reflecting their occurrence after habitat contraction in July (Figure 6.21(ii)).

Table 6.19: Summary of canonical correspondence analysis of benthic invertebrate community and environmental data from the River Glen

	Eigenvalues				**Cumulative % variance of species data**				**Cumulative % variance of species-environ. relation**			
Axis	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
ALL SITES	0.167	0.131	0.057	0.048	12.5	22.3	26.6	30.2	34.8	62.2	74.0	84.0
ALL SITES (covariable)	0.067	0.047	0.037	0.037	6.9	11.8	15.7	19.5	29.6	50.6	67.2	83.5

The original CCA (Figure 6.20) highlighted the primary importance of site-specific variability in determining community composition, with water depth and wetted width proving most influential; this analysis found no significant difference in axes scores between months ($p \geq 0.09$). Repetition of the analysis with site as a covariable (Figure 6.21) highlighted the importance of flow velocity and temperature in determining community composition, with both depth and width remaining relatively constant over time. There was, however, considerable overlap between months and spatial variability remained particularly important in June, when samples formed largely site-specific clusters that spanned the length of axis 2 (Figure 6.21(i)).

6.8.2 Canonical correspondence analysis: hyporheic community

Data was available for five environmental variables: temperature, pH, conductivity, DO concentration and DO % saturation. Preliminary analysis showed the DO measures to be autocorrelated (variance inflation factors ≥27.9) and concentration was therefore excluded. Monte Carlo tests indicated that pH did not contribute to the explanatory power of the model ($F = 1.39$, $p = 0.1180$) and this variable was therefore also excluded. No arch effect was observed and detrending was therefore not required.

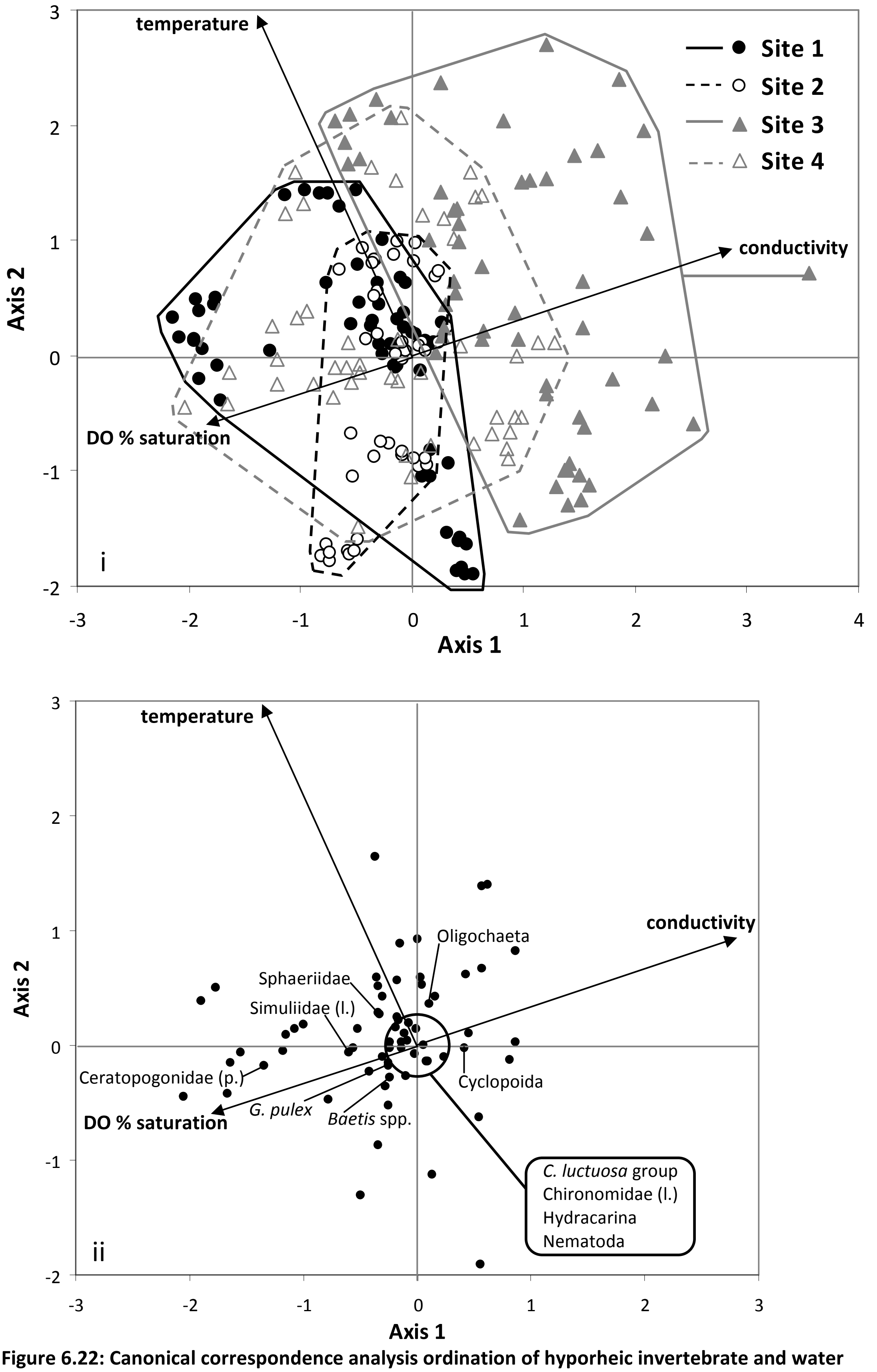


Figure 6.22: Canonical correspondence analysis ordination of hyporheic invertebrate and water chemistry data. Spatial variability: i) sample-environment biplot. Key: circles = West Glen; triangles = East Glen; ii) species-environment biplot.

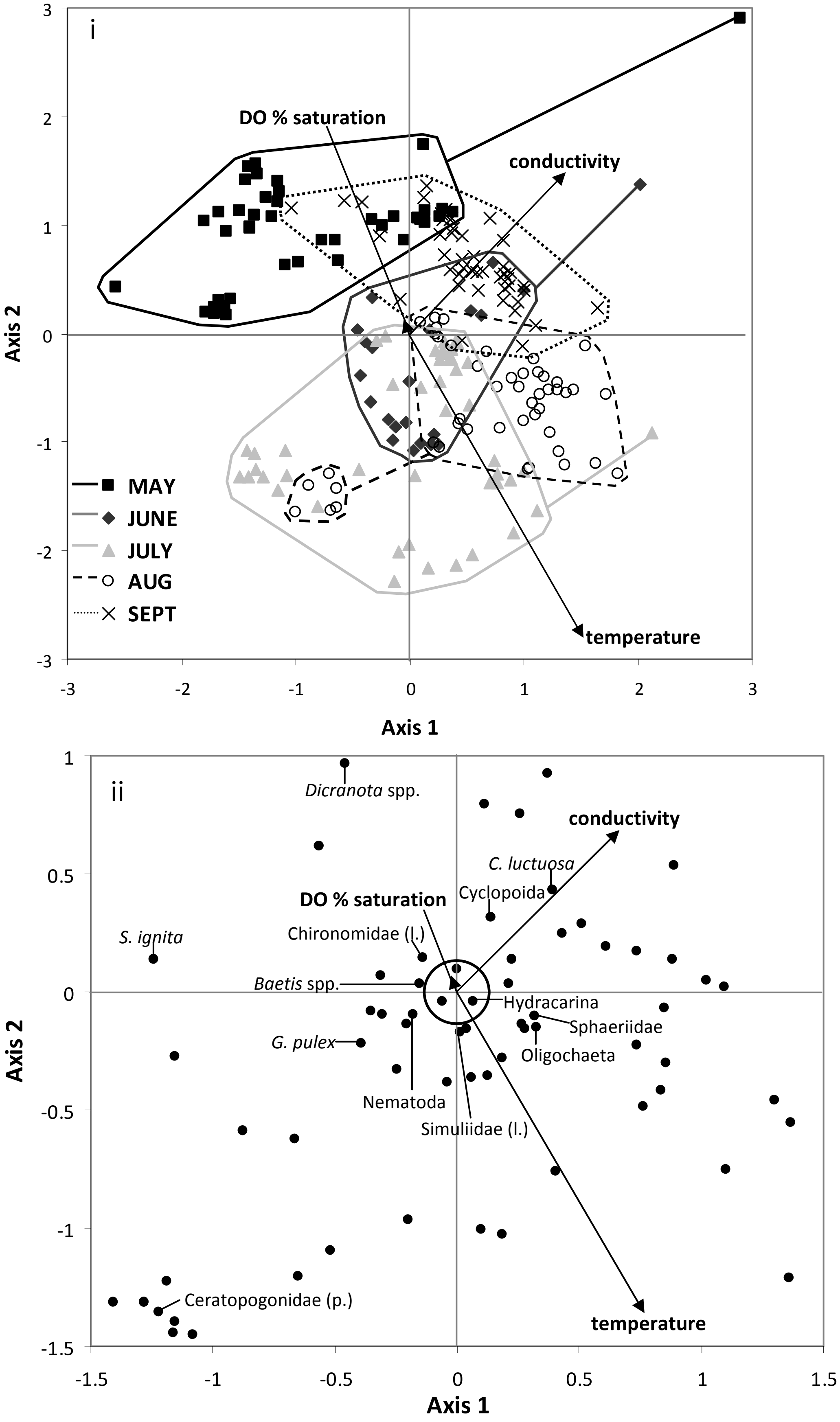


Figure 6.23: Canonical correspondence analysis ordination of hyporheic invertebrate and water chemistry data, with site as a covariable. Temporal variability: i) sample-environment biplot; ii) species-environment biplot.

Spatial variability

Monte Carlo tests found both the first canonical axis and the trace to explain a significant amount of the variation in the dataset (*F*-ratio = 5.184, *p* = 0.002 and *F*-ratio = 3.598, *p* = 0.002 respectively). The contribution of all three variables to the explanatory power was similar (*F*-ratios = 3.07-4.05, *p* = 0.001). However, eigenvalues were very low, indicating weak environmental gradients (Table 6.20).

Axis 1 explained only 2.5 % of the variation in species data and was significantly correlated with all variables. Axis 1 scores differed between sites ($F_{3, 202}$ = 39.914, *p* <0.001); *post-hoc* multiple-comparison tests indicated that this was solely due to higher scores at site 3 (Figure 6.22(i)). Considerable overlap was observed between all other sites, with site 2 samples forming a distinct cluster near the axis origin and site 4 samples being widely distributed. Many common taxa were ubiquitous and plotted at the centre of the ordination, whilst Ceratopogonidae pupae, which occurred only at site 4, and Simuliidae larvae, which were not observed at site 3, plotted in the negative quadrants (Figure 6.22(ii)). The high scoring axis 1 outlier (Figure 6.22(i)) had unusually high conductivity whilst the community featured the highest densities of both Chironomidae larvae and Cyclopoida copepods recorded, explaining the positive score of the latter taxon.

Axis 2 explained only 1.3 % of the species data variation but had a particularly strong correlation with temperature. Despite considerable overlap, axis 2 scores did differ between sites ($F_{3, 202}$ = 7.814, *p* <0.001; Figure 6.22(i)), and as for axis 1, this spatial variability reflected a distinction between site 3 and all other sites. Mean temperature was moderate at site 3, but was particularly variable and samples with the highest axis 2 scores were characterised by higher temperatures, low DO availability, and particularly depauperate communities. Most taxa plotted towards the centre of axis 2, whilst those taxa with the most extreme scores were represented by single individuals (Figure 6.22(ii)).

Temporal variability

Within-site clustering observed on the CCA ordination examining spatial variability suggested the importance of temporal change, and this was examined further by including site as a covariable (Figure 6.23). All three variables made a significant contribution to the explanatory power of the model, although significance was marginal for DO % saturation (*F*-ratio = 1.54, $p = 0.05$). Monte-Carlo tests also indicated the significance of both axis 1 (*F*-ratio = 3.740, $p = 0.008$) and the trace (*F*-ratio = 2.768, $p = 0.002$), although low eigenvalues indicated weak environmental gradients (Table 6.20).

Axis 1 explained only 1.8 % and axis 2 a further 1.5 % of the community variation (Table 6.20); both axes were most strongly correlated with temperate and also had significant correlations with conductivity. Temporal change was significant on both axis 1 ($F_{4, 201} = 30.743$, $p < 0.001$) and axis 2 ($F_{4, 201} = 109.329$, $p < 0.001$). Axis 1 scores were low in May, moderate in June and July and high in August and September (Figure 6.23(i)); this pattern could not be related to any single environmental variable, and appeared to be primarily influenced by seasonal declines in insect abundance, hence the positioning of Chironomidae, *Baetis* spp. and *S. ignita* in the top left quadrant (Figure 6.23(ii)). Axis 2 scores decreased between May and July then rose in each subsequent month, closely reflecting changes in hyporheic water temperature. An outlying group of August samples reflected unusually low conductivity at site 3, whilst the invertebrate communities of these samples were diverse (Figure 6.23).

Spatiotemporal variability

Comparison of the two ordinations (Figures 6.22 and 6.23) indicated that temporal changes in community composition were more pronounced than spatial differences; this contrasts with the distinct site-specific assemblages observed for the benthic community. Temporal change was particularly apparent at individual sites 1 and 2; notably, reference to the raw data revealed the site 1 low-scoring axis 1 cluster (Figure 6.22(i)) to reflect high hyporheic abundance of several predominantly benthic taxa (particularly Simuliidae larvae) in June. The significant correlations between axis scores and temperature, as well as the positioning of insect taxa away from the origin, suggested that temporal changes were largely seasonal rather than related to hydrological variability.

Table 6.20: Summary of canonical correspondence analysis of hyporheic invertebrate community and environmental data from the River Glen

	Eigenvalues				**Cumulative % variance of species data**				**Cumulative % variance of species-environ. relation**			
Axis	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
ALL SITES	0.099	0.052	0.049	0.464	2.5	3.8	5.1	16.8	49.3	75.4	100	0
ALL SITES (covariable)	0.066	0.051	0.026	0.432	1.8	3.3	4.0	16.1	46.0	81.8	100	0

6.9 Spatial variability in invertebrate habitat

To help explain use of the hyporheic zone by benthic invertebrates, two ‘stable’ habitat parameters that can affect hyporheic invertebrate community composition are investigated: the sediment grain size distribution and the direction and strength of hydrologic exchange (aim 2, objective 5). Whilst both of these variables are, in fact, temporally variable (in particular during spate events), they are relatively constant compared to the hydrological and water quality parameters measured each month.

6.9.1 Sediment composition

To facilitate comparison with previous studies, the grain size distribution of McNeil samples collected from each site (see section 4.6.2) was expressed using a range of sediment size classes, from <63 µm to >8 mm (Table 6.21). Two sediment size classes differed between sites, <63 µm and 125-150 µm, both of which were similar at sites 2-4 but higher at site 1 ($F \geq 5.756$, $p \leq 0.021$). On-site observations at site 1 indicated that sample points 1 and 4 were situated in a clay-rich area, whilst point 2 was located in a more heterogeneous area where clay was only present at a depth of ~20 cm (see Appendix 6 for sampling point locations). A clay layer was also present near the base of all site 3 samples; the percentages for the finest size classes presented in Table 6.21 being underestimates due to aggregation of finer sediments during oven drying. Site 2 was characterised by the highest percentage of larger (>2 mm) grains.

Table 6.21: Grain size distribution of sediment samples from River Glen sites 1-4

	% sediment in each size class											
	Site 1 sample areas			**Site 2 sample areas**			**Site 3 sample areas**			**Site 4 sample areas**		
Sediment size class	**1**	**2**	**4**	**1**	**2**	**4**	**1**	**2**	**4**	**1**	**2**	**4**
<63 µm	4.9	1.3	4.6	0.7	0.6	0.4	0.6	1.0	0.6	0.4	0.4	0.4
63-125 µm	6.9	2.3	1.9	1.7	2.2	1.3	1.4	2.0	1.7	0.7	0.8	0.9
125-250 µm	9.5	3.8	9.5	2.5	3.4	1.6	2.7	3.4	5.0	2.3	2.1	2.0
250-500 µm	**14.6**	**12.1**	**13.2**	5.1	6.6	3.9	7.1	**25.1**	**12.9**	**12.9**	**10.9**	**11.8**
0.5-1 mm	**18.1**	**13.4**	**12.7**	7.7	**12.2**	7.9	8.7	**17.9**	9.3	7.2	**13.0**	**15.3**
1-2 mm	**14.9**	9.9	**17.2**	**13.5**	**11.8**	**11.0**	7.7	5.5	7.8	5.8	**12.6**	**10.0**
2-4 mm	9.7	8.7	**17.4**	**15.4**	**12.6**	**14.2**	**11.1**	6.6	**10.6**	9.3	**12.8**	**10.3**
4-8 mm	4.6	**14.2**	8.7	**14.4**	**15.8**	**15.9**	**20.4**	**12.9**	**16.8**	**16.1**	**16.3**	**12.7**
>8 mm	**16.8**	**35.3**	8.7	**39.0**	**34.9**	**43.8**	**40.3**	**25.7**	**35.4**	**45.2**	**31.1**	**36.6**

Sediments not characterised at sampling area 3 of any site. Size classes comprising >10 % of the sediment weight in bold; dominant size class is highlighted.

Pearson correlation coefficients were calculated to assess the effects of relatively constant sediment composition on hyporheic community metrics (Table 6.22). Invertebrate abundance had weak negative correlations with the three finest sediment size classes, this being significant for the 125-250 µm fraction (Table 6.22). Taxon richness was also negatively correlated with these fine sediment classes, this also being most pronounced for the 125-250 µm fraction. Equally, there was a highly significant positive correlation between the number of taxa in the hyporheic zone and the percentage of grains >8 mm. Community dominance appeared unaffected by the grain size distribution, although additional analyses comparing dominance with *cumulative* percentages of sediment below successive thresholds indicated a weak, non-significant but consistent pattern of higher dominance values in sediments with a greater proportion of fine (particularly <1 mm) grains (Table 6.22). Analysis of data from individual depths did not reveal any other significant patterns.

Table 6.22: Pearson correlation coefficients between sediment composition and hyporheic invertebrate community metrics

Sediment size class	**Abundance**[1]	**Taxon richness**[1]	**Dominance**[2]	**Cumulative sediment size class**	**Dominance**[2]
<63 µm	-0.206	-0.369**	0.037	**<63 µm**	0.035
63-125 µm	-0.201	-0.204	-0.056	**<125 µm**	0.031
125-250 µm	-0.256*	-0.422**	0.102	**<250 µm**	0.060
250-500 µm	0.090	0.000	0.236	**<500 µm**	0.175
0.5-1 mm	0.176	-0.037	0.150	**<1 mm**	0.179
1-2 mm	-0.019	-0.246	-0.126	**<2 mm**	0.129
2-4 mm	-0.198	-0.222	-0.166	**<4 mm**	0.093
4-8 mm	0.046	0.179	0.025	**<8 mm**	0.132
>8 mm	0.122	0.345**	0.132	**-**	-

[1]Combined from all hyporheic depths; [2]Berger-Parker dominance index, mean of all hyporheic depths; *p ≤0.05; ** p ≤0.01

6.9.2 Hydrologic exchange

Monthly mini-piezometer data were collected from July onwards at sites 2-4, but measurements were only taken in September at site 1. The installed equipment was simplistic, and was intended to allow determination the direction of exchange and rough estimation of its strength; it is not, however, appropriate to attempt calculation of the precise vertical hydraulic gradient.

Site 4 measurements indicated strongly downwelling water in all months; such readings could reflect the presence of sediments with low hydraulic conductivity between the piezometer base and the sediment surface (Boulton, 2007b). However, site 4 commonly dries during the summer months, in part due to transmission losses through the streambed (Rushton and Tomlinson, 1999), and these readings are therefore considered accurate. Site 2 has also experienced recent streambed drying due to karstic sinkholes and was predominantly downwelling, although the strength of this exchange generally weakened during the study, with some upwelling water being recorded in September.

Hydrologic exchange was also weak- to moderately-downwelling at perennial site 1 in September, although functioning of the mini-piezometers may have been compromised by clay layers (see section 6.9.1). Similarly, stable to moderately-upwelling water was recorded at perennial site 3 (in what is considered a gaining reach; Rushton and Tomlinson, 1999), but the strength of exchange may have been underestimated due to the presence of clay in some parts of the substrate.

6.10 Discussion

In this section, the results of the sampling campaign on the River Glen are described in relation to the thesis aims (section 1.2). Environmental conditions are considered as potential environmental stressors, then the benthic invertebrate community response to each identified stressor is considered, with particular focus on the use of the hyporheic refugium. Both habitat-related and disturbance-related factors that may have limited refugium use are emphasized.

6.10.1 Identification of potential environmental stressors

The first aim of this chapter (section 6.2) was to identify changes in surface hydrology, hydrologically-mediated environmental conditions and related biotic factors with the potential to stress benthic invertebrates.

Variation in surface hydrology in long-term context

The hydrological conditions on the Glen included a series of high-flow events between the May and June sampling dates (Figure 6.2). These were potentially adverse hydrological conditions for benthic invertebrates. However, flow duration analysis (Figures 6.3 and 6.4) demonstrated that the highest discharges recorded during the study were unexceptional and higher-magnitude spates occurred during the preceding winter (data not shown); the invertebrate community should therefore be adapted to resist such events (Lytle and Poff, 2004). Flow duration analysis also indicated that the lowest flows were greater than is typical, although the hydrograph (Figure 6.2) showed that site 4 experienced short-duration streambed drying in both late July and early September. Hydrological conditions therefore remained moderate compared with long-term average conditions, but localised streambed drying may have been a direct invertebrate stressor at site 4 in later months.

Effect of flow variability on instream habitats

Discharge data alone is insufficient to characterise effects of flow variability on benthic invertebrate habitat, and localised development of adverse conditions was observed. Firstly, flow velocities were low at site 3 (Figure 6.5(ii)), particularly from June when localised ponding occurred; these low velocities reduced habitat heterogeneity and were associated with low oxygen and high fine sediment concentrations, both of which limit habitat suitability and reduce survival for many benthic taxa (Wood and Armitage, 1997; Connolly *et al*., 2004). Secondly, width and depth were particularly low at site 4 in July (Figure 6.5(i) and (iii)). A low water volume can increase the influence of external factors (e.g. insolation and groundwater; Dewson *et al*., 2007a), but minor increases in temperature and associated reductions in dissolved oxygen availability in July were unlikely to have had biotic effects (Murdoch *et al*., 2000). Of greater potential importance was the reduction in habitat availability (Table 6.5), since habitat contraction can concentrate benthic invertebrates into a smaller submerged area (Fritz and Dodds, 2004; Dewson *et al*., 2007b). This habitat reduction was followed by complete loss of surface water, which represents a critical threshold (*sensu* Boulton, 2003) after which impacts on aquatic fauna are severe (e.g. Smock *et al*., 1994; Fritz and Dodds, 2004); this temporary loss of surface water is likely to explain particularly low hyporheic oxygen availability in August (September data not available).

Potential effects of flow variability on biotic interactions

Whilst no common non-insect taxon experienced significant temporal changes in overall abundance following habitat contraction at site 4, a notable fourfold increase in *Gammarus pulex* abundance was observed between June and July. *G. pulex* is a highly competitive taxon which can potentially influence community composition through both competition and predation (Dick *et al*., 1995), however, population densities remained much lower than reported by some studies (Mortensen, 1982; Crane, 1994), and as such the taxon is unlikely to have caused a significant intensification of biotic interactions (e.g. predation). Similarly, the Hydracarina occurred at particularly high densities in July at site 4, however Hydracarina primarily consume meiofauna and so are unlikely to have impacted upon macroinvertebrate populations (Cassano *et al*., 2002). Whilst many other taxa also occurred at high abundance at site 4 in July, total invertebrate densities did not approach values recorded in May, when habitat availability was high. Habitat contraction and the concurrent increase in population densities may therefore have caused only a moderate increase in the strength of biotic interactions.

6.10.2 Benthic community response to hydrological variability

The second aim of this chapter (section 6.2) was to examine benthic community responses to factors identified as potential stressors, and community composition in the benthic sediments is considered in this section.

Temporal change in benthic community composition

Multivariate ordinations indicated that environmental and benthic community differences were generally more pronounced between sites than between months, suggesting that temporal change in hydrological conditions had little impact on community composition. Considering the series of small spates, such events are recognised as disturbance events which can have pronounced impacts on benthic invertebrate communities (Death, 2008). However, spate magnitude and duration are determinants of detrimental impacts and some previous studies have shown low-intensity spates to have little impact on benthic communities (Robinson *et al*., 2004). In the River Glen, only the Chironomidae were observed to decline significantly in abundance between May and June, and this is may reflect seasonal adult emergence (Learner and Potter, 1974); similarly, the Simuliidae experienced a considerable decline in abundance, which could either reflect displacement by high flows or adult emergence (Table 6.11).

At the other hydrological extreme, reduced flow at site 4 in July coincided with increases in the abundance of most common taxa, indicating concentration of stable populations into a contracting habitat area. In addition, taxon richness at site 4 peaked in July following exposure of marginal benthic sediments, due to the arrival of Coleoptera associated with exposed sediments (i.e. *Agabus didymus, Helophorus brevipalpis*; Stubbington *et al*., 2009b). Despite habitat availability returning to 'normal' at site 4 in August, severe reductions were observed in both taxon richness and TIA, the latter reflecting considerable reductions in a diverse range of taxa including the Hydracarina (>99 % decline), *Baetis* spp. (>99 %), *G. pulex* (94 %), Chironomidae (89 %) and *Asellus aquaticus* (88 %). The severity of these reductions almost certainly relate to short-duration complete streambed drying in late July. The reduced abundances generally declined further in September, which is assumed to relate to the second drying event between August and September sampling. Interestingly, reductions in both richness and abundance increased community diversity due to a disproportionate impact on dominant taxa (Death, 2008; Mesa, 2010). Only two common taxa appeared unaffected by the loss of surface water: the Oligochaeta, a group including many species with physiological adaptations to drying

(Kenk, 1949; Williams, 2006), and *Bithynia leachii*, a prosobranch gastropod which can seal its operculum to prevent water loss.

6.10.3 Benthic invertebrate use of the hyporheic zone

In this section, evidence for the hyporheic refuge hypothesis (Williams and Hynes, 1974) is examined for each condition previously identified as a potential stressor.

Use of the hyporheic zone following the spate

Sampling in the current investigation was conducted at monthly intervals, with discharge data indicating that samples were collected several days after the spate disturbances (Figure 6.2). Even field studies specifically targeting spates are restricted to sampling in the days following an event (e.g. Dole-Olivier *et al*., 1997; Olsen and Townsend, 2003), thus limiting the extent to which conclusions can be drawn regarding refugium use *during* high flows. In the Glen, whilst the spates affected all sites, these events did not have detectable detrimental impacts on the benthic community, with the possible exceptions of the Chironomidae and Simuliidae. The need for migration into the hyporheic refugium was therefore limited to few taxa. Of these taxa, a moderate decline in the benthic abundance of the Simuliidae was accompanied by a significant increase in its hyporheic abundance and in the hyporheic proportion of the total population (Figure 6.19; Table 6.18); this provides tentative evidence of Simuliidae either actively migrating or being passively washed into the hyporheic zone during high flows.

Use of the hyporheic zone during habitat contraction

At site 4, two potential invertebrate stressors were identified: habitat contraction and an associated increase in benthic population densities (and therefore biotic interactions) in July; and recent streambed drying in August and September. In July, an increase in benthic abundance during habitat contraction was accompanied by some rise in hyporheic population densities for many taxa, including Chironomidae, Oligochaeta, *Baetis* spp., Hydracarina and *G. pulex*. However, in no case was this associated with an increase in the hyporheic proportion of the total population. This is unsurprising, since conditions appeared to remain favourable in the contracting habitat, and hyporheic increases are considered passive range extension rather than active refugium use (Wood *et al*., 2010; see section 7.11 and Figure 7.4). Nonetheless, the moderate increases in hyporheic abundance suggested that the hyporheic zone at site 4 was suitable for inhabitation by several benthic taxa.

Use of the hyporheic zone after streambed drying

The hyporheic zone is proposed to act as a refugium during streambed drying due to the retention of free water (Boulton *et al*., 1992). However, previous evidence of this refugial role is equivocal, with some studies noting active migrations to greater depths following drying (Boulton *et al*., 1992; Cooling and Boulton, 1993; Griffith and Perry, 1993), whilst others have observed no increase in invertebrate abundance (Boulton, 1989; Boulton and Stanley, 1995; Del Rosario and Resh, 2000). In the Glen, severe reductions in benthic abundance in August and September were accompanied by declines in the hyporheic abundance of common taxa including Chironomidae, Hydracarina, *G. pulex* and *Baetis* spp. In contrast, the hyporheic abundance of the Oligochaeta remained stable in July and August then increased in September, whilst benthic abundances fell; the associated increase in the hyporheic proportion of the oligochaete population was not, however, significant. Therefore, no conclusive evidence supporting the hyporheic refuge hypothesis was recorded on the Glen, although oligochaetes may have migrated into the hyporheic zone during drying events to enhance survival. This taxon is morphologically suited to interstitial environments and species are commonly tolerant of low oxygen availability (e.g. Extence *et al*., 1987); its capacity to exploit the hyporheic zone refugium is therefore high. Regardless of whether hyporheic abundance and/or hyporheic proportion increased, any taxon that persisted within the hyporheic zone following streambed drying used these sediments to enhance survival, even if refugium use was passive (see section 7.11).

6.10.4 Suitability of the hyporheic sediments as a refugium.

The final objective set for this chapter was to relate spatial variability in hyporheic refugium use to the suitability of the hyporheic sediments as a benthic invertebrate habitat (section 6.2). Since little evidence of refugium use was recorded, conditions that may have lowered the hyporheic zone's refugial capacity are emphasized in the following discussion, with reference to historic flow characteristics, stable habitat variables (sediment grain size distribution, hydrologic exchange) and temporally variable water quality parameters.

Flow regime

Sites with historic perennial and intermittent flow had been selected to allow comparison of refugium use at sites with contrasting historic flow permanence regimes, this being considered a possible influence on hyporheic community composition and refugium use (Hose *et al.*, 2005; Wood *et al.*, 2010). However, relationships between flow permanence and environmental/community parameters were scarce and significant differences were instead tributary- or site-specific. It is therefore more appropriate to disregard the flow permanence distinction and consider site-specific flow regime characteristics and their potential impacts on refugial capacity.

Site 1 is perennial and retained flowing water; hydrologic exchange with the hyporheic zone should therefore have delivered oxygen and organic matter to the hyporheic sediments, promoting refugial capacity (Jones *et al*., 1995a; Brunke and Gosner, 1997). Site 2 was considered as intermittent but has dried recently only due to sporadic development of karst sinkholes (C. Extence, pers. comm.); this reach had previously been considered perennial (Maddock *et al*., 1995) and during the study had similar flow characteristics to adjacent site 1. Site 3 is also perennial, but may become ponded for long periods (Maddock *et al*., 1995), as occurred between June and September. As current velocities decline, fine sediment can be deposited and clog interstitial spaces, reducing hydrologic exchange and detrimentally affecting hyporheic water quality (Brunke and Gosner, 1997; Boulton, 2007a); therefore, the suitability of the hyporheic zone as a refugium at site 3 may have been low. Site 4 is truly intermittent and typically dries during the summer months. Much previous research into the hyporheic zone refugium has focussed on intermittent sites (e.g. Clifford, 1966; Boulton *et al*., 1992; Clinton *et al*., 1996; Fenoglio *et al*., 2006), reflecting the particular challenges faced by benthic invertebrates in such environments rather than suggesting that the hyporheic zone functions as a particularly effective refugium. In fact, the ability of the hyporheic zone to support benthic invertebrates following drying relies on the retention of free water, and refugial capacity is severely reduced if water is lost (Gagneur and Chaoui-Boudghane, 1991; Boulton and Stanley, 1995). No measurements of hyporheic water content were taken during this study; however, complete water loss was observed to a depth of >35 cm in 2009, indicating that transmission losses through the streambed can also cause hyporheic drying.

Sediment characteristics

Previous studies have noted negative relationships between the percentage of fine sediment in the substrate and community metrics (Richards and Bacon, 1994; Olsen and Townsend, 2003; Weigelhofer and Waringer, 2003), since fine sediments limit movement of invertebrates through interstices and also weaken hydrologic exchange, thus reducing water quality (Brunke, 1999; Sarriquet *et al*., 2007). The proportion of fine sediment in bulk samples from the Glen were high at all sites compared with the fourth-order gravel-bed stream studied by Olsen and Townsend (2003) but comparable with the third-order sandstone stream of Weigelhofer and Waringer (2003) and accordingly, significant negative correlations between fine sediment classes and taxon richness were observed in the Glen (Table 6.22). The proportion of fine sediments was particularly high at site 1 (Table 6.21), with field observations indicating that clay layers affected two of four sampling areas at this site and were also present in parts of site 3, and suitability of the hyporheic zone as a refugium is likely to have particularly low in these areas.

Hydrologic exchange

The direction and strength of vertical hydrologic exchange (i.e. upwelling or downwelling water) is a major influence on the hyporheos (Boulton and Stanley, 1995; Davy-Bowker *et al*., 2006). It has also been identified as influencing migrations of benthic invertebrates during disturbance events, with upwelling water impeding movement into the hyporheic zone whilst downwelling water facilitates migrations (Dole-Olivier *et al*., 1997). In the Glen, strongly downwelling water should have promoted migrations into the hyporheic sediments at site 4, whilst upwelling water may have limited benthic invertebrate use of deeper sediments at site 3.

Water quality

Water quality was lower in the hyporheic zone compared with the surface channel at all sites, with significantly lower dissolved oxygen, higher temperatures and slightly higher conductivity being recorded and localised anoxia occurring at sites 3 and 4. Such observations are typical, with hyporheic oxygen availability being particularly low in upwelling zones (such as at site 3; Jones *et al*., 1995a) and following streambed drying (as at site 4; e.g. Smock *et al*., 1994). The hyporheic zone has therefore rarely been reported to function as a refugium from poor water quality (but see Jeffrey *et al*., 1986). In addition, several previous studies have noted negative correlations between community metrics and hyporheic dissolved oxygen concentrations (Boulton *et al.*, 1997; Franken *et al.*, 2001).

6.10.5 Disturbance-related determinants of refugium use

In addition to environmental variability, refugium use can also be influenced by disturbance related parameters. Both Lancaster (2000) and Boulton *et al*. (2004), for example, attributed the failure of the hyporheic zone to act as a high-flow refuge to spate magnitude being too low to elicit a behavioural response; this reason is also given here to explain the lack of response to the Glen spates. Similarly, previous research considering refugium use following flow reduction has suggested that no migrations occurred due to conditions remaining favourable in the benthic sediments (James *et al*., 2008); again, such an explanation may be given for benthic invertebrates largely remaining in the surface sediments following habitat contraction at site 4. At the other extreme, disturbance magnitude may increase too quickly to allow a behavioural response, as has been observed in relation to high-magnitude spates (Imbert and Perry, 1999; Gayraud *et al*., 2000). Whilst a rapid rate of change has not previously been cited as determining refugium use following streambed drying, the flashy flow regime of the East Glen (and consequent rapid shifts between wet and dry conditions) may have limited refugium use.

6.11 Summary

Spate events, habitat contraction during low flows and streambed drying were all identified as potential stressors of the benthic community. However, closer inspection of benthic community composition indicated that both spates and habitat contraction had only localised and taxon-specific effects. Accordingly, little use of the hyporheic refugium was expected and evidence of active migrations was restricted to the Simuliidae during the spate. Streambed drying was identified as the major potential trigger of hyporheic refugium use. However, evidence of active refugium use during drying was limited to the Oligochaeta, whilst the hyporheic zone may have functioned as a passive refugium for a diverse range of taxa. A range of environmental and disturbance-related parameters have been discussed individually regarding their potential to limit the refugial capacity of the hyporheic zone. Whilst the failure of the hyporheic zone to act as refugium can sometimes be attributed to the overriding influence of an individual variable (Boulton, 1989 – high temperature; Smock *et al*., 1994 – anoxia; Olsen and Townsend, 2003 – high proportion of fine sediments), in many cases the combined influence of environmental variables outlined above determines refugium use (or lack thereof). Interplay between factors will be further discussed in Chapter 7.

6. Invertebrate community response to flow variability: the River Glen

6.1 Introduction

This chapter considers the responses of invertebrates in the benthic and hyporheic sediments to variation in surface flow on the River Glen. Discharge in the Glen is groundwater-dominated, but variation in the underlying geology (see Chapter 3) makes the flow regime responsive to rainfall in some reaches. During the study period (May-September 2008), hydrological conditions included a series of low-magnitude high-flow events, a two-month low flow period, habitat contraction and short-duration streambed drying. In total, 312 invertebrate samples were collected over five months, comprising 80 each from the benthic sediments and from hyporheic depths of 10 cm and 20 cm, and 72 samples from a depth of 30 cm (this difference being due to difficulties installing sampling wells). Temporal changes in benthic and hyporheic community composition are examined in relation to surface flow variability and consequent changes in habitat availability and water quality. Particular consideration is given to the occurrence of benthic invertebrates in the hyporheic zone, and use of this habitat is related to both temporally variable environmental factors (e.g. hydrology and water quality) and relatively stable habitat parameters (hydrologic exchange and sediment composition).

6.2 Aims and objectives

This chapter examines changes in the composition and distribution of invertebrate communities in the benthic and hyporheic sediments of the River Glen during a period of variable surface flow. The specific aims and objectives of this chapter are as follows:

Aim 1: Identify hydrological conditions and related changes in both environmental and biotic variables with the potential to stress benthic invertebrates.

Objectives

1. Examine variation in surface hydrology, using discharge data and by measuring site-specific instream variables.
2. Set the hydrological conditions experienced in context using long-term data.
3. Determine changes in habitat availability resulting from variation in surface flow.
4. Investigate changes in water quality parameters related to surface flow variation.
5. Use multivariate analysis to determine the principal environmental gradients.
6. Analyse changes in the abundance of taxa with the potential to influence the strength of biotic interactions (such as predation and competition) in the benthic sediments.

Aim 2: Examine invertebrate community responses to identified potential stressors, including changes in the use of the hyporheic zone by benthic invertebrates.

Objectives

1. Investigate temporal change in invertebrate community composition in the benthic and hyporheic zones using community metrics and multivariate analysis.
2. Identify temporal changes in the abundance of common benthic taxa in the surface sediments and the hyporheic zone.
3. Analyse temporal changes in the proportion of the total (benthic + hyporheic) community resident in the hyporheic zone.
4. Examine relationships between environmental conditions and community metrics to infer drivers of community change.
5. Investigate spatial variability in the suitability and use of the hyporheic refugium, with reference to historic flow regime, stable habitat parameters and temporally variable environmental factors.

6.3 Meteorological conditions and hydrological response

To address the first aim (objective 2), the prevailing meteorological conditions during the study are presented. Air temperature and rainfall are described and compared with long-term averages (LTA), and streamflow response to precipitation is examined using rainfall and discharge data (objective 1).

6.3.1 Air temperature

Air temperature showed a sharp increase between mid-April and mid-May, a second more gradual increase until July/August, then a decline in September (Figure 6.1; Table 6.1; also see section 4.8.1). The 12-hourly minimum temperatures exceeded zero in mid-May, then remained >5 °C for the rest of the study (Figure 6.1); 12-hourly maxima exceeded 24 °C every month from May onwards, but such high temperatures were only common in late July (Figure 6.1). Monthly mean temperatures increased each month between April and July, peaking in August (Table 6.1).

Table 6.1: Air temperature in the Glen catchment, April to September 2008, in comparison with long-term average conditions

	Air temperature (°C)						
	Maxima			**Minima**			**Mean***
	Daily absolute[§]	**Daily mean[§]**	**LTA**, Daily mean**	**Daily absolute[§]**	**Daily mean[§]**	**LTA**, Daily mean**	**Monthly**
April	21	12.9	11.7	-1.9	3.4	4.0	7.65
May	25.3	19.1	15.4	1	8.3	6.7	12.6
June	24.6	19.2	18.3	5.5	9.9	9.7	14.3
July	28.1	22.0	21.0	8	12.2	11.9	16.4
August	24.9	21.0	20.9	8.1	13.4	11.8	16.8
Sept	20.7	18.0	17.7	4.6	9.6	9.8	13.6

[§] 12-hour minimum/maximum values from Monks Wood (TL200801, 40 km to the south)

* calculated using weekly MORECS data (NERC, 2009); Figure 6.1 and section 4.8.1 provide details.

** Long-term averages (LTA, 1971-2000) for Waddington, 40 km to the north (Met Office, 2009c)

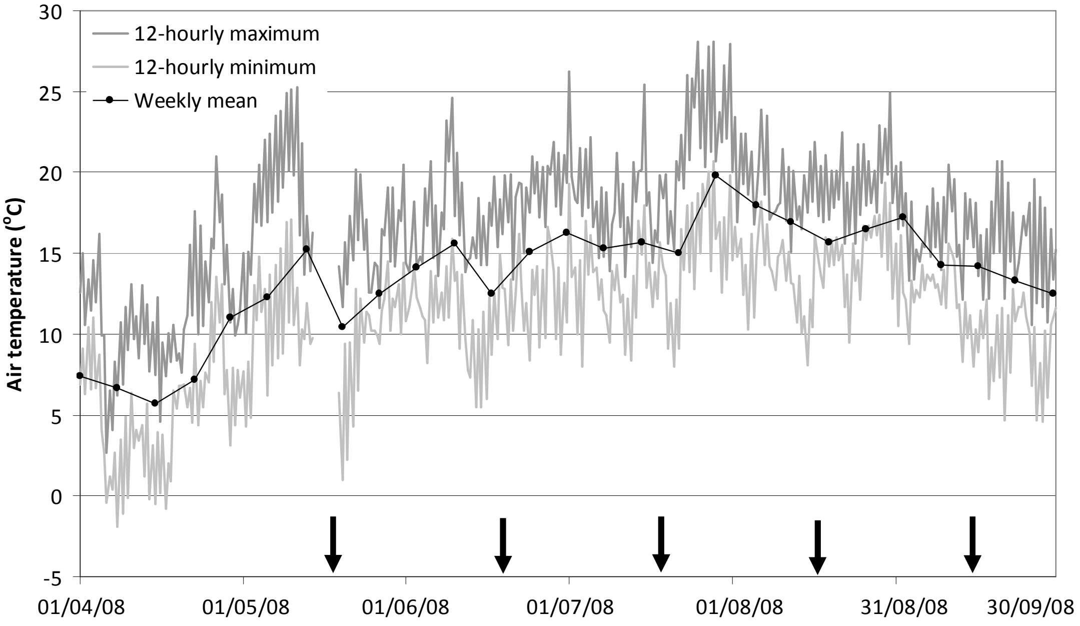


Figure 6.1: 12-hour minimum and maximum air temperature at Monks Wood and weekly mean air temperature for the Glen catchment region, April-September 2008. Arrows indicate sampling dates.

At a regional scale (the Midlands, East Anglia and Eastern England), 2008 daily mean minimum and maximum temperatures were ~2 °C above the LTA (1971-2000) in May, and close to the LTA between June and September (Met Office, 2009d). Comparison of the Monks Wood data with long-term (1971-2000) records from Waddington (SK988643, 40 km to the north of the Glen at a similar elevation; Met Office 2009c) indicated that mean daily maximum temperature in the Glen catchment exceeded the LTA throughout the study, by 3.7 °C in May and by ≤1 °C in later months (Table 6.1). Mean daily minimum temperatures were also above the LTA between May and August, and close to the LTA in September (Table 6.1).

6.3.2 Rainfall and streamflow response

Following recharge of the underlying aquifer by above-average rainfall between January and April 2008 (BADC, 2009), streamflow responded to minor precipitation inputs in May and June (Figure 6.2). This response was particularly pronounced in early June, when streamflow temporarily increased by between four- and ten-fold above baseflow on three successive occasions in the West Glen (Figure 6.2(i)), and by 18- to >100-fold on two occasions in the East Glen (Figure 6.2(ii)). These events were followed by rainfall below the 1980-2008 average in June and July (BADC, 2009) during which baseflow was stable on the West Glen at Little Bytham and declined (briefly to zero) at Manthorpe on the East Glen (Figure 6.2). Exposure of marginal areas was observed at site 4 in mid-July and complete streambed drying occurred in late July and again in early September at site 4. August rainfall was almost twice the 1980-2008 monthly average (104.3 mm compared with 58.9 mm; BADC, 2009), resulting in some small increases in streamflow. The cause of the streamflow fluctuations observed on the West Glen hydrograph (Figure 6.2(i)) during baseflow conditions are not known but may be due to abstractions associated with local quarrying activity (Ian Gray, Environment Agency, pers. comm.).

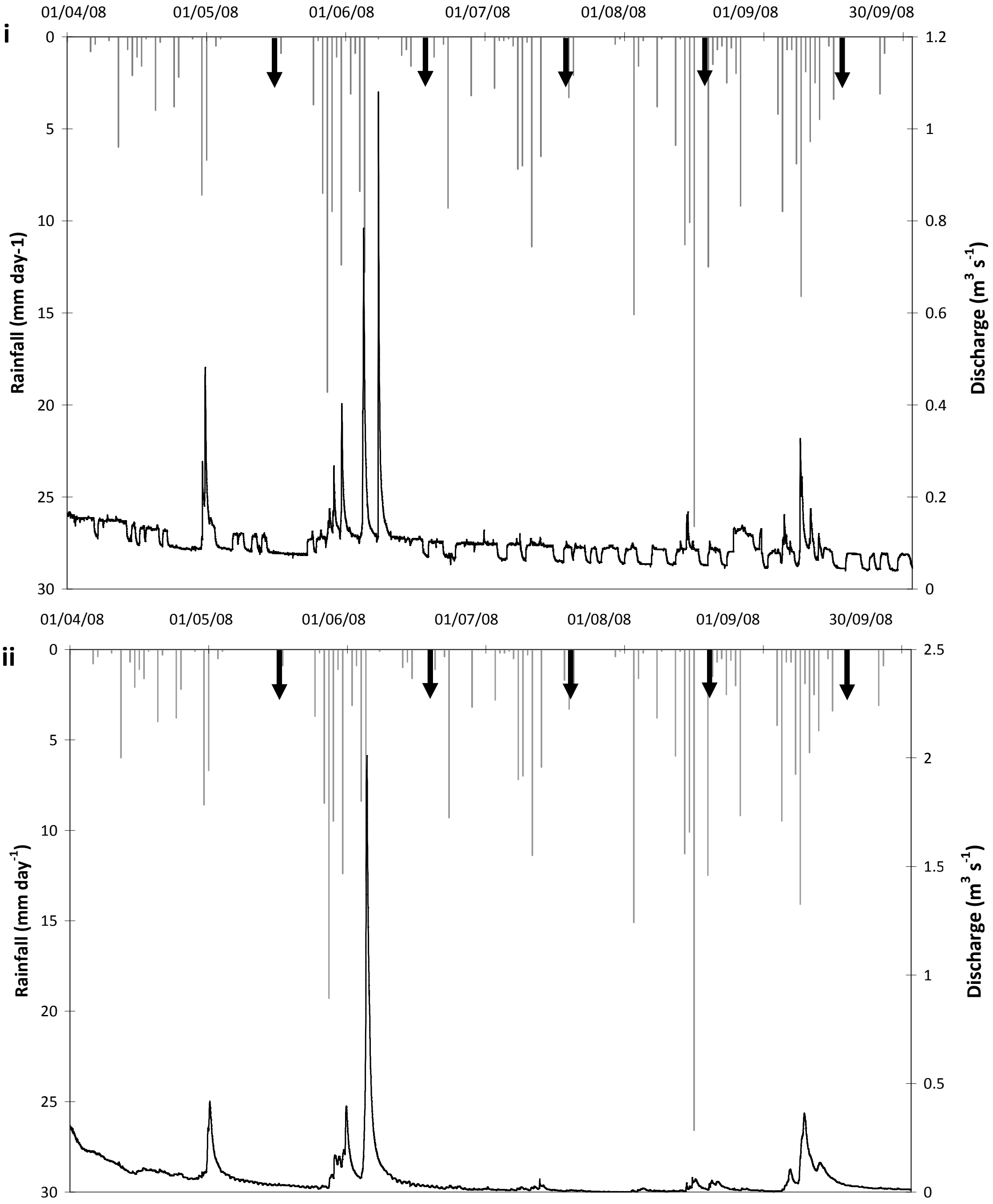


Figure 6.2: Daily rainfall at Carlby and 15-min resolution hydrographs for: i) the River West Glen at Little Bytham; ii) the River East Glen at Manthorpe (see Figure 3.6), April- September 2008. Arrows indicate sampling dates.

6.3.3 Flow duration analysis

Flow duration analysis was undertaken (Figure 6.3; Figure 6.4) and associated indices calculated (Table 6.3) to set the high and low flow conditions observed during the study year (water year ending 30th September 2008) in the context of the long-term (1981-2009) average conditions (see section 4.8.2).

On the East Glen at Manthorpe (~1 km downstream of site 4, Figure 3.6), the steep slope in the upper region of the FDC (Figure 6.3 (i)) and the low values of high-flow indices (Q_1, Q_5, Q_{10} and Q_{30}; Table 6.2) indicated that spate events were of a below-average magnitude and duration during the study year. However, the median value (Q_{50}), which provides a measure of 'average' discharge, was similar in 2007-08 to the LTA (Table 6.2). At the low-flow end of the curve (Figure 6.3(ii)), the rate at which

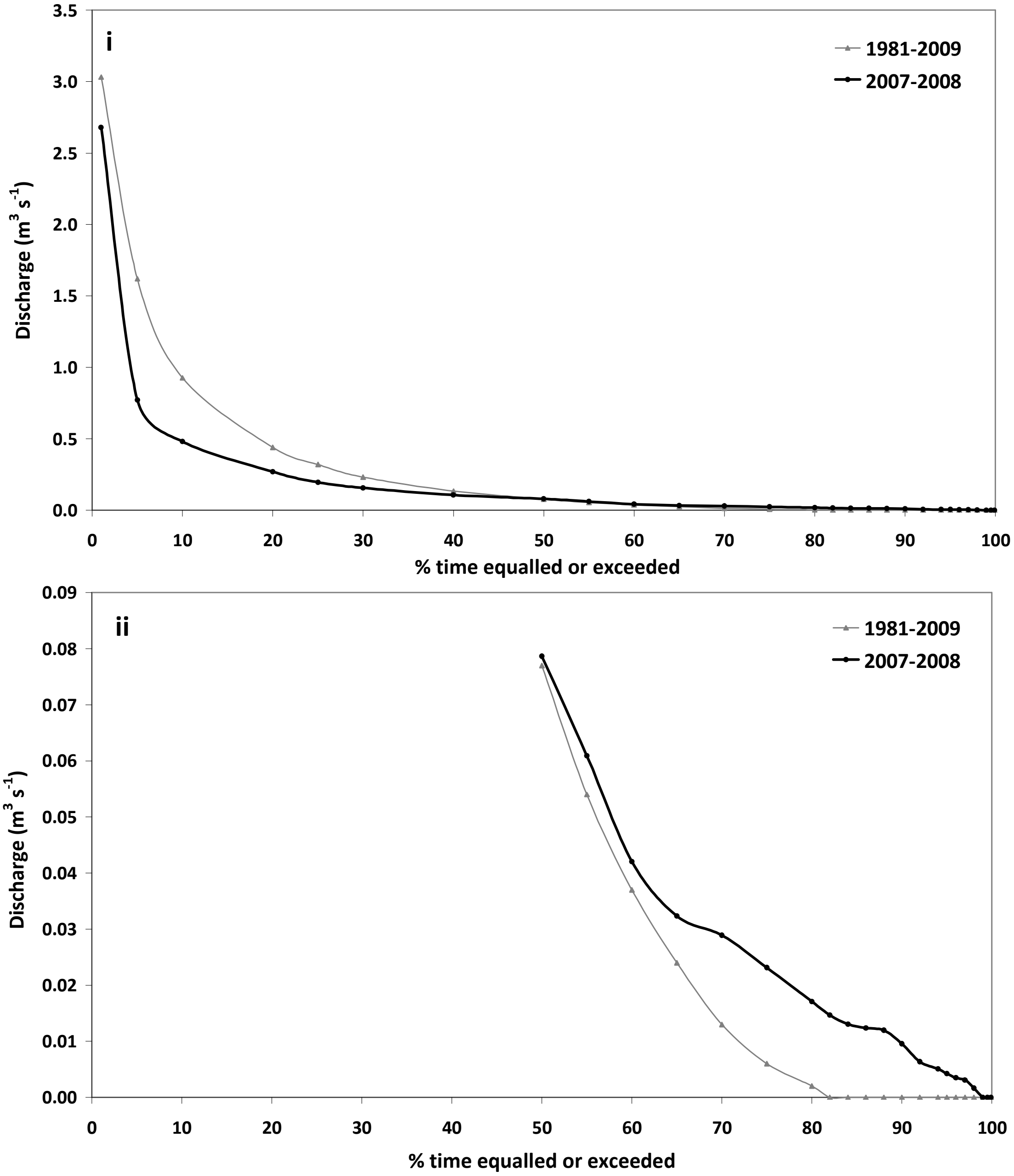


Figure 6.3: Flow duration curves for the River East Glen at Manthorpe. Mean daily discharge equalled or exceeded: i) 0-100 % time; ii) 0-50 % time.

discharge declined was more variable and slower than average, and zero-flow conditions occurred for an unusually short period (Q_{99} = 0 compared with Q_{90} = 0 for the LTA; Table 6.2).

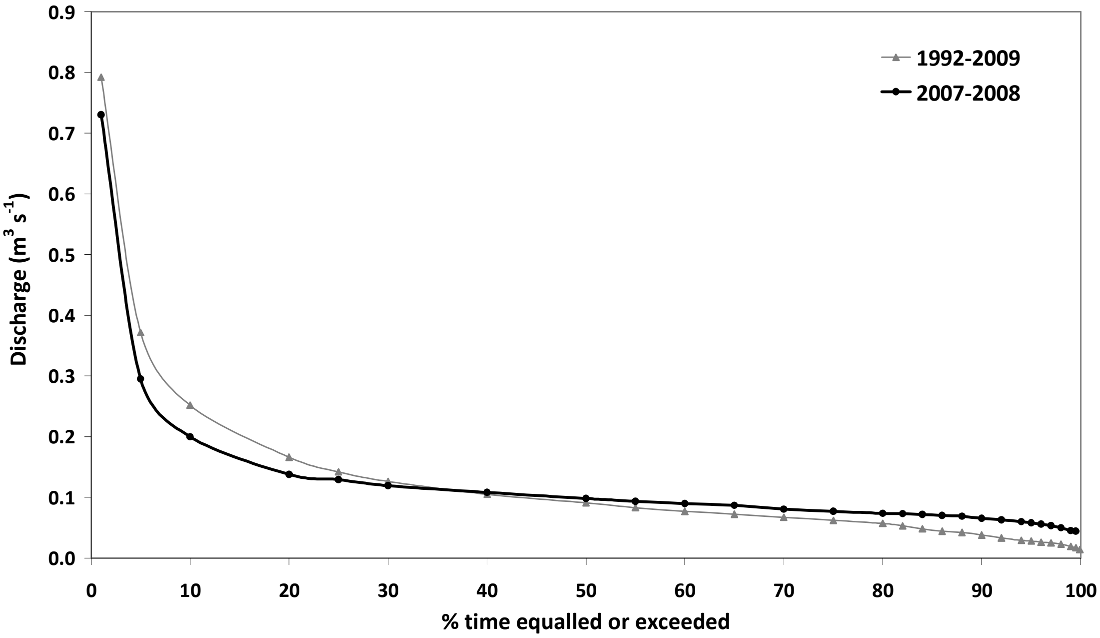


Figure 6.4: Flow duration curves for the River West Glen at Little Bytham. Mean daily discharge equalled or exceeded 1-100 % time.

Table 6.2: Indices derived from flow duration analysis, indicating discharge during the study year in comparison with the long term average

	Mean daily discharge (m^3 s^{-1})			
	Little Bytham (West Glen)		Manthorpe (East Glen)	
Index	**1992-2009**	**2007-2008**	**1981-2009**	**2007-2008**
Q_1	0.792	0.735	3.032	2.680
Q_5	0.372	0.295	1.620	0.770
Q_{10}	0.252	0.200	0.926	0.480
Q_{30}	0.126	0.119	0.231	0.156
Q_{50}	0.091	0.098	0.077	0.079
Q_{90}	0.038	0.065	0.000	0.010
Q_{95}	0.028	0.058	0.000	0.004
Q_{99}	0.019	0.045	0.000	0.000

On the West Glen at Little Bytham, the slope in the upper region of the FDC was only slightly steeper than is typical and median and high flow indices were similar to the LTA, whilst the low flow end of the curve and related indices remained above average (Figure 6.4; Table 6.2). However, all high flow indices were only slightly reduced in comparison with the LTA and, as is typical, flow never fell to zero. Comparison of the East and West Glen FDCs indicated that the East Glen experiences a flashier, more variable flow regime, including both streambed drying and higher magnitude spates (Figure 6.3; Figure 6.4).

6.4 Spatiotemporal variability in environmental conditions

Temporal changes in instream variables are considered (aim 1). First, the effects of discharge variability on the hydrological parameters measured instream are examined (objective 1), then the effects of these changes on submerged habitat availability are determined (objective 3). Associated changes in water quality parameters are also considered (objective 4), then principal components analysis is used to identify the main environmental gradients in the dataset (objective 5).

6.4.1 Variation in surface hydrology

In addition to the continuous discharge data obtained from the Environment Agency (Figure 6.2), water depth and mean flow velocity (at 0.6x depth) were measured each month at each sampling point, to determine the effects of discharge variability on the environment inhabited by the invertebrate community. Wetted width was also determined *post hoc* by application of depth measurements to cross-sectional channel profiles. Following description of temporal variability in these parameters, consequent changes in habitat availability are examined.

Surface water depth, flow velocity and wetted width

Spatially, mean depth was higher at both West Glen sites compared with the East Glen ($F_{1, 14} = 30.256$, $p < 0.001$; Table 6.4). Of the five sampling occasions, depth peaked in June and was lowest in September ($F_{1.827, 27.411} = 4.691$, $p = 0.020$; Figure 6.5(i); Table 6.3). The interaction with depth was significant for site ($F_{12, 48} = 8.487$, $p < 0.001$) and the overall pattern only reflected conditions at sites 1 ($F_{1.738, 5.213} = 6.726$, $p = 0.038$) and 3 ($F_{1.454, 4.363} = 6.837$, $p = 0.049$). At site 4, depth was considerably lower in July than in any other month ($F_{1.738, 5.213} = 6.726$, $p = 0.038$), whilst depth did not change significantly at site 2 (Figure 6.5(i)).

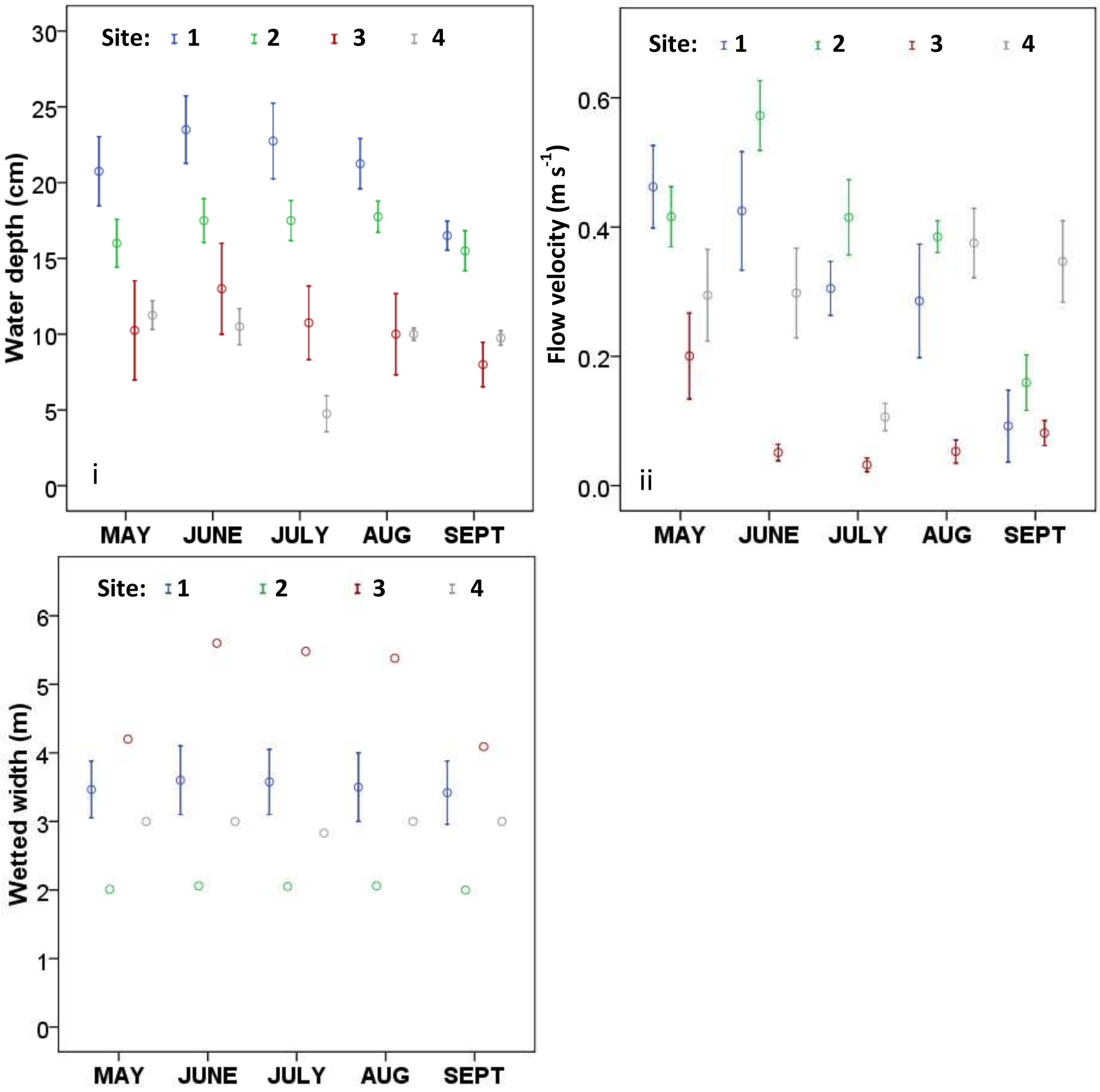


Figure 6.5: Mean ± 1 SE temporal change in hydrological variables: i) water depth; ii) mean flow velocity; iii) wetted width (replicates only available from site 1).

Considering all months, velocities were lower at site 3 than at any other site ($F_{3,\,12}$ = 21.218, p <0.001; Table 6.4). Considering all sites, velocities peaked in May and June and were lowest in September ($F_{2.155,\,32.328}$ = 5.516, p = 0.007; Table 6.3; Figure 6.5(ii)). The interaction with velocity was significant for site ($F_{12,\,48}$ = 6.283, p <0.001) and the overall pattern of temporal change was only observed at site 1 ($F_{1.840,\,5.521}$ = 6.121, p = 0.041). At site 2, the pattern was similar but with particularly high velocities in June ($F_{1.417,\,4.251}$ = 8.594, p = 0.036); at site 3, velocities were higher in May than in any subsequent month, but temporal change was not significant; and at site 4, velocities were lower in July than in other months ($F_{1.801,\,5.402}$ = 18.117, p = 0.004; Figure 6.5(ii)).

Mean wetted width was particularly high at site 3 and lowest at site 2 (Table 6.4; Figure 6.5(iii)). Mean values varied little between months, from 3.2 ± 0.4 m in May and September to 3.6 ± 0.60 m in June (Table 6.3; Figure 6.5(iii)), due to a combination of channel morphology (particularly on the West Glen; Appendix 8) and low variation in depth, which although significant, occurred within a range of <4.0 cm (Table 6.3). Considering individual sites, width at site 3 declined each month between June (5.6 m) and September (4.0 m), whilst at site 4 widths were very similar in all months (3.0 m) except July (2.8 m; Figure). Statistical analyses of these spatiotemporal changes in width were hampered by insufficient replicates.

Table 6.3: Temporal change in surface water hydrology of the River Glen, May to September 2008

Variable	**May**	**June**	**July**	**August**	**Sept**	**Temporal change***
Hydrological variables						
Surface water depth (cm)	14.6 ± 1.5	16.1 ± 1.6	13.9 ± 2.0	14.8 ± 1.5	12.4 ± 1.1	**
Mean flow velocity (m s^{-1})	0.34 ± 0.04	0.34 ± 0.06	0.26 ± 0.06	0.28 ± 0.04	0.17 ± 0.04	**
Wetted width (m)	3.2 ± 0.38	3.6 ± 0.60	3.5 ± 0.59	3.5 ± 0.56	3.2 ± 0.37	ns

Values presented as the mean ± 1 SE of all samples; *n* = 16 in all months for depth and velocity, *n* = 5 in all months for width. *One-way RM ANOVA tests; ** indicates *p* <0.01, ns indicates *p* >0.05.

Table 6.4: Spatial differences in surface water hydrology at River Glen sites 1-4.

Variable	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Spatial Change***
Surface water depth (cm)	21 ± 1.0	17 ± 0.6	10 ± 1.1	9 ± 0.6	**
Mean flow velocity (m s^{-1})	0.3 ± 0.04	0.4 ± 0.04	0.08 ± 0.02	0.3 ± 0.03	**
Wetted width (m)	3.5 ± 0.03	3.9 ± 0.04	5.0 ± 0.33	3.0 ± 0.03	ns

Values presented as the mean ± 1 SE of all samples; *n* = 20 at all sites for depth and velocity, *n* = 10 at site 1 and *n* = 5 at sites 2-4 for width. *Two-way RM ANOVA tests; ** indicates *p* <0.01, ns indicates *p* >0.05

6.4.2 Submerged habitat availability

Width and depth data were applied to cross-sectional channel profiles of each site to investigate the effect of discharge variability on submerged habitat availability (see section 4.5.2). The maximum extent of submerged benthic sediments recorded during the investigation was determined for each site using the cross-sectional levelling survey data and on-site observations regarding the extent of benthic sediments. The percentage of this maximum benthic habitat that was submerged and thus available for invertebrate inhabitation (% maximum submerged benthic habitat; % max. SBH) was then determined for each other month. At sites 1, 2 and 4, a single cross-section was representative of all sampling points, whilst two cross-sections were considered at site 3 due to variation in bed morphology (Appendix 8).

Table 6.5: Temporal change in extent of submerged benthic sediments as a percentage of the maximum recorded

	Submerged % of benthic sediments at site:				
Site	**1**	**2**	**3 (1)***	**3 (3)***	**4**
May	100	86.7	100	65.3	100
June	100	100	100	100	89.8
July	100	100	100	88.1	22.6
August	100	100	100	88.1	100
Sept	100	86.7	74.2	59.4	89.8

*** Bracketed numbers refer to the closest sampling point**

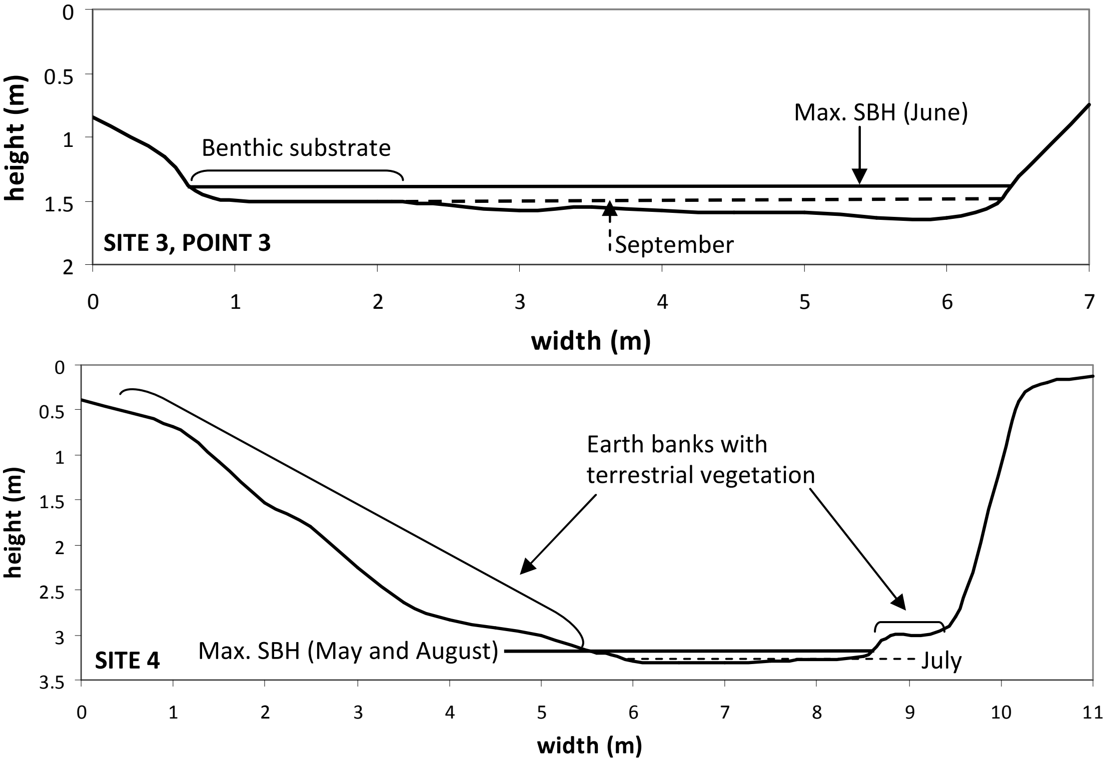


Figure 6.6: Cross-sectional profiles of sites 3 and 4, illustrating changes in the extent of submerged benthic sediments in relation to changes in water depth. Key: Max. SBH = maximum extent of submerged benthic habitat.

A trapezoidal channel shape at sites 1 and 2 resulted in maximum SBH being reached at a low depth, and the % max. SBH therefore remained similarly high in all months (Table 6.5). In contrast, the streambed of site 3 had a gentle cross-slope between steep banks (Figure 6.6) and the availability of submerged habitat was more responsive to changes in depth (Table 6.5). Whilst the streambed at site 4 could also be described as a gentle cross-slope between steep banks (Figure 6.6), depth at this site was very low in July (3 cm, compared to ≥9 cm in all other months), resulting in a dramatic decline in submerged habitat availability (Table 6.5; Figure 6.6).

6.4.3 Water quality

Temporal variability in water quality parameters that may have changed in response to variation in surface flow was examined; monthly mean values of all variables are presented in Table 6.6. Unless otherwise stated, values were comparable at all hyporheic depths, which were therefore pooled prior to analysis. Spatial differences were considered between tributaries, flow permanence groups and sites; for brevity, only significant patterns are described (also see Table 6.7). Also for brevity, non-significant results are not always stated; $p > 0.05$ in all cases.

Dissolved oxygen

DO readings were not obtained at sites 2 and 4 in June or September; these months were therefore excluded from analysis unless otherwise specified. Both concentration and saturation were determined (and are detailed in Tables 6.6 and 6.7), but these measures followed the same spatial and temporal patterns and therefore only % saturation is described in detail.

DO levels in surface water were considerably lower at East Glen sites compared with the West Glen ($F_{1, 14} = 11.382$, $p = 0.005$; Table 6.7). DO availability was higher in May, June and September, and relatively low in July and August ($F_{1.234, 18.510} = 5.614$, $p = 0.023$; Table 6.6; Figure 6.7(i)). The interaction with site was significant ($F_{3.604, 14.415} = 12.033$, $p < 0.001$), and June and September data could be included in analysis of sites 1 and 3. At site 1, DO peaked in June and was lowest in July and August ($F_{4, 12} = 161.020$, $p = 0.001$); similarly, at site 2, DO was higher in May compared with July and August ($F_{2, 6} = 190.176$, $p < 0.001$); at site 3, DO was particularly low in August and peaked in September ($F_{1.333, 3.998} = 19.419$, $p = 0.010$); and at site 4, DO availability was lowest in July but temporal change was not significant (Figure 6.7(i)).

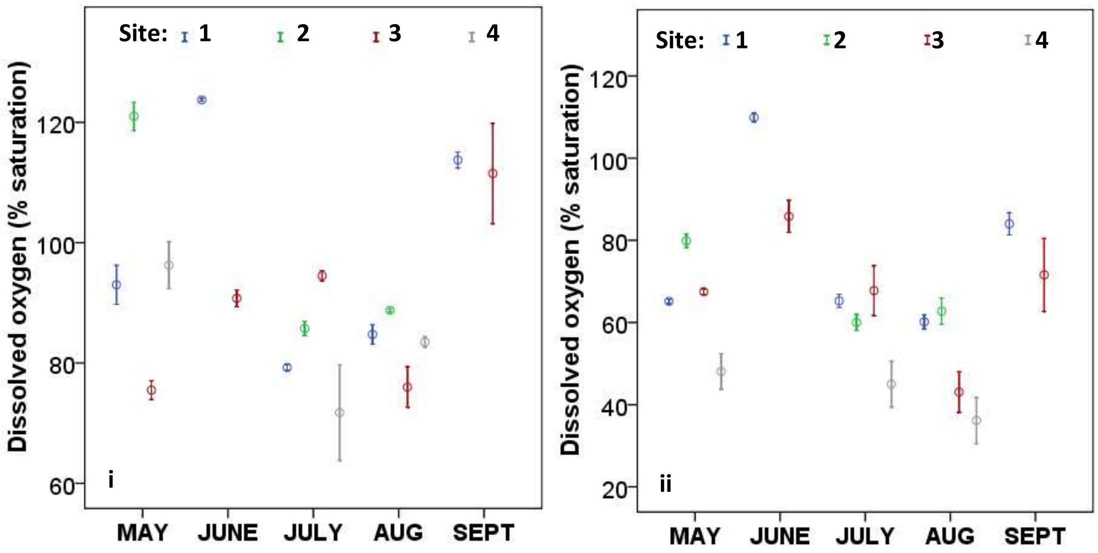


Figure 6.7: Mean ± 1 SE temporal change in dissolved oxygen (% saturation): i) surface water; ii) hyporheic water. No data is available for site 2 or site 4 in June or September.

Mean DO values were significantly lower in hyporheic water (57.6 ± 1.5 %) compared with surface water (87.5 ± 2.0 %; $F_{1, 58}$ = 72.647, p <0.001) and decreased slightly with increasing hyporheic depth. Spatially, hyporheic DO levels were particularly low at site 4 ($F_{3, 40}$ = 13.142, p <0.001; Table 6.7). Hyporheic DO peaked in June, was low in July and particularly low in August ($F_{2, 86}$ = 17.019, p <0.001; Table 6.6; Figure 6.7(ii)).

The interaction with site was significant for hyporheic DO ($F_{6, 80}$ = 4.412, p = 0.001), and data were available for all months at sites 1 and 3. At site 1, DO was particularly high in June and was lowest in August ($F_{4, 44}$ = 158.365, p <0.001); a similar pattern was seen at site 3, although the June peak was less prominent and the August dip more pronounced ($F_{1.937, 21.306}$ = 13.322, p <0.001); at site 2, DO values were considerably higher in May compared with July and August ($F_{2, 14}$ = 18.336, p <0.001); and at site 4, DO declined gradually between May and August and was very low in the latter month (mean 36.2 ± 5.6 %), but temporal change was of only marginal significance ($F_{2, 22}$ = 2.953, p = 0.073).

Water temperature

Surface water temperatures ranged between 11.3 ^{o}C at sites 1 and 3 in May to 16.2 ^{o}C at site 3 in July, whilst mean values were particularly high at site 4 ($F_{3, 12}$ =

131.466, p <0.001; Table 6.7). Temporally, mean temperatures were lowest in May then increased each month to a July/August peak ($F_{1.978, 29.671} = 39.171$, p <0.001; Table 6.6; Figure 6.8(i)). The interaction with temperature was significant for site ($F_{12, 48} = 209.906$, p <0.001), with slight variations on the overall pattern observed at sites 2, 3 and 4 ($F_{\geq 128.535}$, p <0.001). At site 1, lowest temperatures also occurred in May, but this was followed by a June peak ($F_{1.534, 4.601} = 400.039$, p <0.001).

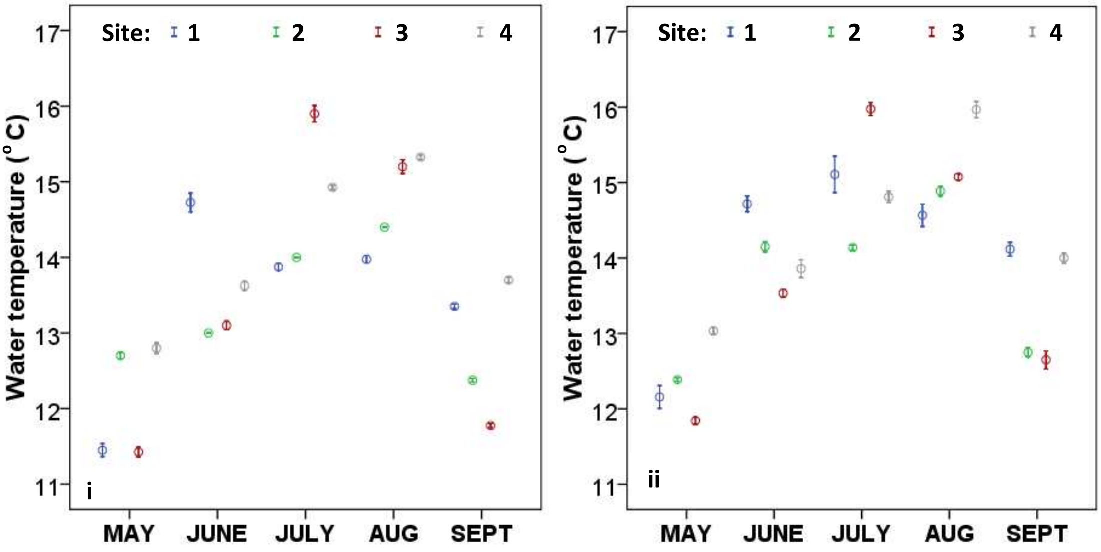


Figure 6.8: Mean ± 1 SE temporal change in water temperature (°C): i) surface water; ii) hyporheic water.

Mean temperature was higher in hyporheic water (14.0 ± 0.08 °C) than in surface water (13.6 ± 0.14°C; $F_{1, 58} = 19.902$, $p = 0.013$), but similar at all hyporheic depths. Significant differences in hyporheic temperature were observed between sites, with similarly high temperatures at sites 1 and 4, and significantly lower temperatures at sites 2 and 3 ($F_{3, 40} = 17.674$, p <0.001; Table 6.7). Considering all sites, patterns of temporal variability in hyporheic temperature reflected those reported for surface water and were highly significant ($F_{2.559, 110.042} = 150.053$, p <0.001; Table 6.6; Figure 6.8(ii)). Whilst the interaction with water temperature was significant for site ($F_{8.589, 114.524} = 49.044$, p <0.001), site-specific patterns of change differed only slightly in statistical significance and in the timing of peak values.

Particulate organic carbon

Surface water POC concentrations were lowest at site 1 and higher at all other sites, particularly site 4, but spatial variation was not significant ($F_{3, 8} = 0.675$, $p = 0.591$; Table 6.7). Considering all sites, surface POC concentrations decreased and became less variable between July and September ($F_{2, 6} = 5.427$, $p = 0.045$; Figure 6.9(i); Table 6.6). The interaction with POC was not significant for any spatial parameter and monthly declines occurred at all sites.

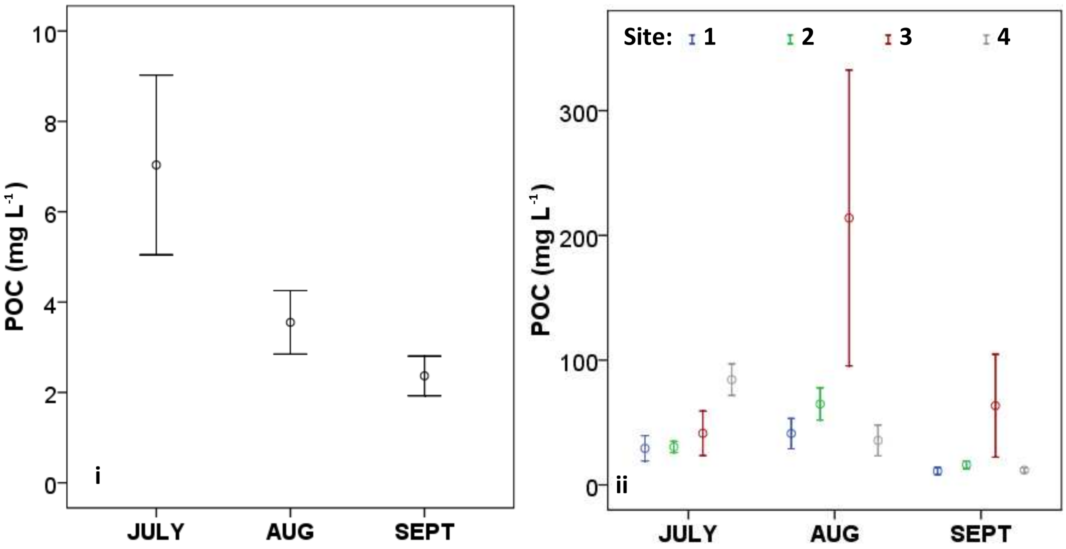


Figure 6.9: Mean ± 1 SE temporal change in particulate organic carbon concentrations (mg L^{-1}): i) surface water (pooled data); ii) hyporheic water.

POC concentrations in surface and hyporheic water could not be compared due to different sampling techniques, but values were similar at all hyporheic depths. Hyporheic concentrations were very high at site 3 compared to all other sites but values were statistically comparable ($F_{3, 7} = 1.231$, $p = 0.368$; Table 6.7). Overall, POC concentrations were high (but very variable) in August and lowest in September ($F_{1.165, 11.651} = 8.977$, $p = 0.009$; Figure 6.9(ii); Table 6.6), with a significant interaction being observed with site ($F_{6, 14} = 3.884$, $p = 0.017$). Temporal change was not, however, significant at any individual site.

Fine sediment

Surface water fine sediment concentrations were considerably higher at site 3 compared with all other sites, but spatial variability was not significant ($F_{3, 8} = 1.090$,

p = 0.407; Table 6.7). Concentrations were high and variable in August, due in part to a site 3 outlier, and lowest and in September; temporal change was not significant ($F_{2, 6}$ = 0.682, p = 0.541; Table 6.6; Figure 6.10(i)). Interactions with fine sediment were not significant for any spatial parameter and insufficient data were available for site-specific analyses. Concentrations in surface and hyporheic water were not compared due to different sampling techniques, but were comparable at all hyporheic depths. As in surface water, hyporheic concentrations were much higher at site 3 than at other sites but this was not significant ($F_{3, 8}$ = 1.185, p = 0.375; Table 6.7); neither was temporal change significant (Table 6.6; Figure 6.10(ii)).

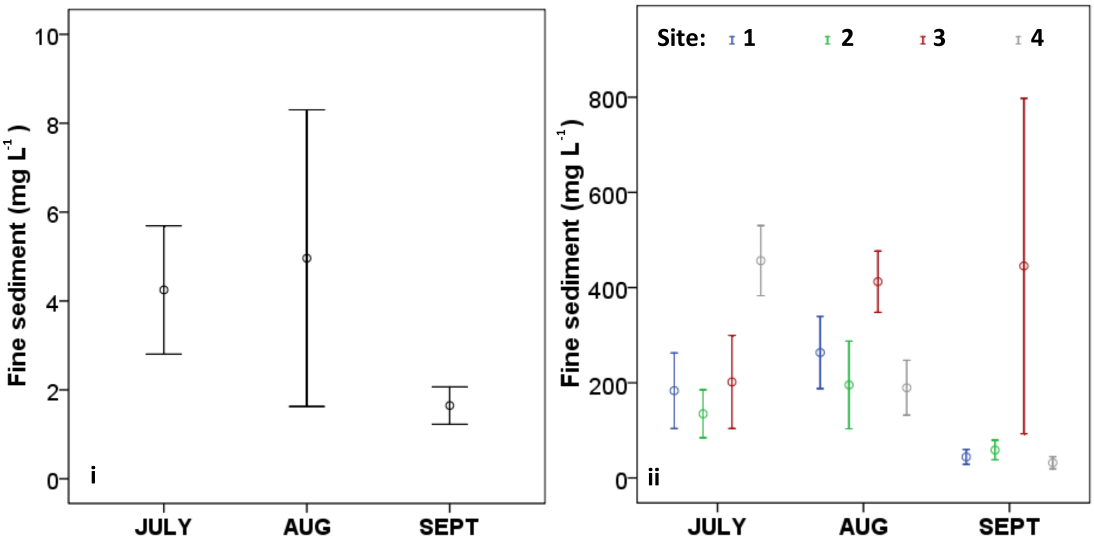


Figure 6.10: Mean ± 1 SE temporal change in fine sediment concentrations (mg L^{-1}): i) surface water; ii) hyporheic water (a September site 3 outlier has been normalised from 2898 mg L^{-1} to be in line with other values).

Nitrate and phosphate

Mean surface water nitrate concentrations were considerably higher at West Glen sites compared with the East Glen ($F_{1, 2}$ = 250.416, p = 0.004); this pattern was also seen in hyporheic water (Table 6.7). Surface and hyporheic nitrate concentrations were very similar ($F_{3, 7}$ = 0.038, p = 0.989). However, whilst temporal change was not significant overall or at any individual site in surface water, in hyporheic water concentrations were lowest in June then peaked in August and remained high in September ($F_{4, 28}$ = 8.525, p <0.001; Table 6.6).

Surface water phosphate concentrations were also higher on the West Glen compared with East Glen ($F_{1, 2}$ = 230.677, p = 0.004; Table 6.7); temporal change was not significant (Table 6.6). Concentrations were comparable in surface water and at all hyporheic depths ($F_{3, 11}$ = 0.264, p = 0.850). Spatial variations in hyporheic phosphate concentrations were equivalent to those reported for surface water. Considering all sites, mean hyporheic concentrations were lowest in May then increased to a peak in August ($F_{4, 40}$ = 10.491, p <0.001; Table 6.6); patterns varied spatially but temporal change was not significant at any individual site.

Conductivity and pH

Conductivity was high on the River Glen (overall mean 818 ± 5.4 µS cm^{-1}). Whilst changes in mean values were spatiotemporally significant, these changes were too minor to be of ecological relevance and they are therefore not examined in detail (but see Tables 6.6 and 6.7). However, it is worth noting that values were slightly higher in hyporheic water (833 ± 3.8 µS cm^{-1}) compared with surface water (818 ± 5.4 µS cm^{-1}), and that values were particularly high at site 3 (surface water mean 879 ± 6.6 µS cm^{-1}; hyporheic mean 911 ± 7.6 µS cm^{-1}; peak 1066 µS cm^{-1}; Table 6.7).

Significant spatial and temporal variability was also observed in pH, but mean values were moderate in all months in both surface and hyporheic water (Table 6.6 and 6.7); detailed analysis of this variable is therefore not required. However, it is of note that numerous particularly high values (8.7-9.1) were recorded across all sites in both surface and hyporheic water in July (Table 6.6).

Table 6.6: Temporal change in physicochemical measures of surface and hyporheic water in the River Glen, May to September 2008

Variable	**Surface or hyporheic**	**May**	**June**	**July**	**August**	**Sept**	**Temporal change**
DO (mg L^{-1})	Surface	9.6 ± 0.59	9.0 ± 0.77	7.3 ± 0.24	7.1 ± 0.16	9.4 ± 0.49	**
	Hyporheic	5.43 ± 0.25	8.03 ± 0.38	4.55 ± 0.29	3.49 ± 0.27	5.45 ± 0.43	**
DO (% saturation)	Surface	96.4 ± 4.4	107.3 ± 6.3	82.8 ± 2.8	83.3 ± 1.5	112.6 ± 3.9	*
	Hyporheic	63.8 ± 2.0	97.9 ± 3.2	59.5 ± 2.6	49.4 ± 2.7	77.8 ± 4.7	**
Water temperature (^{o}C)	Surface	12.1 ± 0.17	13.6 ± 0.18	14.7 ± 0.21	14.7 ± 0.15	12.8 ± 0.20	**
	Hyporheic	12.4 ± 0.08	14.1 ± 0.08	15.1 ± 0.12	15.1 ± 0.10	13.4 ± 0.11	**
Fine sediment (mg L^{-1})	Surface	-	-	4.2 ± 1.4	5.0 ± 3.3	1.6 ± 0.4	ns
	Hyporheic	-	-	240 ± 50	480 ± 230	450 ± 90	ns
POC (mg L^{-1})	Surface	-	-	7.0 ± 2.0	3.6 ± 0.7	2.4 ± 0.4	*
	Hyporheic	-	-	47.8 ± 9	91.1 ± 37	26.5 ± 12	**
Nitrate (mg L^{-1})	Surface	3.2 ± 1.63	1.7 ± 0.88	2.7 ± 1.25	4.3 ± 1.02	4.4 ± 1.01	ns
	Hyporheic	3.2 ± 0.88	1.6 ± 0.70	3.1 ± 0.56	5.0 ± 1.39	4.6 ± 0.65	**
Phosphate (mg L^{-1})	Surface	0.49 ± 0.21	0.42 ± 0.15	0.62 ± 0.20	0.76 ± 0.09	0.77 ± 0.30	ns
	Hyporheic	0.21 ± 0.07	0.38 ± 0.10	0.65 ± 0.09	0.71 ± 0.06	0.61 ± 0.14	**
pH	Surface	8.1 ± 0.03	8.2 ± 0.02	8.3 ± 0.19	7.8 ± 0.06	8.1 ± 0.02	*
	Hyporheic	8.0 ± 0.04	8.1 ± 0.05	8.3 ± 0.07	7.5 ± 0.07	8.0 ± 0.03	*
Conductivity (µS cm^{-1})	Surface	816 ± 12.6	812 ± 12.9	794 ± 12.6	818 ± 6.9	847 ± 11.4	**
	Hyporheic	845 ± 9.5	847 ± 9.4	804 ± 10.5	830 ± 6.6	864 ± 8.9	**

Values presented as the mean ± 1 SE of all samples. In each month, n = 16 for surface water and n = 44 for hyporheic water (comprising n = 16 from 10 cm and 20 cm, n = 12 from 30cm), with the following exceptions: surface DO (mg L^{-1} and % saturation), n = 8 in June and n = 7 in September; hyporheic DO (mg L^{-1} and % saturation), n = 24 in June and September; hyporheic conductivity, n = 36 in all months; hyporheic nitrate, n = 8 in all months; hyporheic phosphate, POC and fine sediment, n = 11 in all months. Temporal change analysed using one-way RM ANOVA: * indicates p <0 .05; ** indicates p <0.01; ns indicates p >0.05. Key: DO = dissolved oxygen; POC = particulate organic carbon.

Table 6.7: Spatial differences in physicochemical measures of surface and hyporheic water at River Glen sites 1-4.

Variable	Surface or hyporheic	Site 1	Site 2	Site 3	Site 4	Spatial change?
DO (mg L^{-1})	Surface	9.0 ± 0.5	8.9 ± 0.6	7.5 ± 0.3	7.6 ± 0.4	**
	Hyporheic	6.2 ± 0.2	5.6 ± 0.3	5.2 ± 0.3	2.8 ± 0.3	**
DO (% saturation)	Surface	86 ± 2.0	99 ± 4.9	82 ± 2.9	84 ± 4.0	**
	Hyporheic	77 ± 2.5	68 ± 2.3	67 ± 3.0	43 ± 3.0	**
Temperature (°C)	Surface	13.5 ± 0.3	13.3 ± 0.2	13.5 ± 0.4	14.1 ± 0.2	**
	Hyporheic	14.1 ± 0.2	13.7 ± 0.2	13.8 ± 0.2	14.3 ± 0.1	**
Fine sediment (mg L^{-1})	Surface	1.84 ± 0.3	3.53 ± 2.4	7.10 ± 4.0	1.99 ± 0.8	ns
	Hyporheic	163 ± 45	129 ± 37	640 ± 314	226 ± 68	ns
POC (mg L^{-1})	Surface	2.36 ± 0.6	4.32 ± 0.8	4.89 ± 1.0	5.70 ± 3.5	ns
	Hyporheic	26.1 ± 5.8	34.6 ± 8.7	99.2 ± 41.3	41.1 ± 10.9	ns
Nitrate (mg L^{-1})	Surface	4.8 ± 0.8	5.4 ± 0.6	1.3 ± 0.4	1.6 ± 0.8	**
	Hyporheic	6.2 ± 1.0	5.2 ± 0.4	1.7 ± 0.4	1.8 ± 0.4	**
Phosphate (mg L^{-1})	Surface	0.79 ± 0.1	0.85 ± 0.2	0.38 ± 0.1	0.41 ± 0.2	**
	Hyporheic	0.65 ± 0.09	0.86 ± 0.07	0.37 ± 0.08	0.28 ± 0.06	**
pH	Surface	7.9 ± 0.08	8.4 ± 0.08	8.1 ± 0.03	8.1 ± 0.12	*
	Hyporheic	8.1 ± 0.03	8.2 ± 0.08	8.0 ± 0.03	7.7 ± 0.07	**
Conductivity (µS cm^{-1})	Surface	802 ± 5.5	806 ± 7.0	879 ± 6.6	783 ± 8.7	**
	Hyporheic	815 ± 2.5	803 ± 4.6	903 ± 5.1	795 ± 4.8	**

Values given as mean ± 1 SE of all samples. Surface water: n = 20 for each site; hyporheic water: n = 60 at sites 1, 3 and 4 (⅓ from 10, 20, 30 cm) and n = 40 at site 2 (½ from 10, 20 cm), with the following exceptions: surface DO (mg L^{-1} & %), n = 12 for site 2 & 4; hyporheic DO (mg L^{-1}& %), n = 24 for site 2, n = 36 for site 4; surface POC and fine sediment, n = 3; hyporheic POC and fine sediment, n = 9; surface nitrate and phosphate, n = 5; hyporheic nitrate and phosphate, n = 9 for sites 1, 3 and 4 and n = 6 for site 2. Key: DO = dissolved oxygen; POC = particulate organic carbon.

6.4.4 Principal Components Analysis

PCA was used to investigate spatiotemporal variation in environmental conditions in both the surface stream and the hyporheic zone, and to identify the major gradients underlying this variation.

Surface water

PCA ordinations are presented both by month and by site (Figure 6.11(i) and (ii)). The first principal component (PC1) explained 32.6 % of the variance and was strongly correlated with conductivity (Pearson correlation coefficient (PCC) = 0.797, p <0.001) and flow velocity (PCC = -0.804, p <0.001). PC2 explained a further 28.3 % of the variance had significant positive correlations with temperature (PCC = 0.632, p <0.001) and pH (PCC = 0.552, p <0.001). Water depth was similarly correlated with both PC1 (PCC = -0.551, p <0.001) and PC2 (PCC = -0.558, p <0.001).

Although considerable overlap was observed between samples from all months (Figure 6.11(i)), temporal change was significant on both PC1 ($F_{4,75} = 3.199$, $p = 0.018$) and PC2 ($F_{4,75} = 6.462$, $p < 0.001$). PC1 scores were similar between May and August but particularly high in September due to high conductivity and low flow velocities. PC2 scores were particularly high in July but comparable in all other months, reflecting high temperatures and pH at site 4 in July (Figure 6.11(i) and (ii)). Comparison of Figures 6.11(i) and (ii) indicated that environmental conditions were influenced more strongly by spatial variability than temporal variability, and these spatial differences were primarily site-specific. PC1 scores were particularly high at site 3 ($p < 0.001$ compared with other sites), reflecting high conductivity and slow flow velocities. PC2 scores were significantly higher at site 4 than at any other site ($p \leq 0.016$), due to high temperatures and high pH. No other significant differences were recorded between sites, and some sites (particularly adjacent sites 1 and 2 on the West Glen) showed complete overlap (Figure 6.11(ii)).

Hyporheic water

Both PC1 and PC2 had significant correlations with all variables ($p \leq 0.036$), although many relationships were weak (PCC ≥0.148). PC1 explained 46.7 % of the variance and was strongly correlated with both oxygen parameters (PCC = -0.876-0.880, $p < 0.001$). PC2 explained a further 17.1 % of the variance and was strongly correlated with conductivity (PCC = 0.808, $p < 0.001$) and temperature (PCC = -0.680, $p < 0.001$; Figure 6.12).

Despite considerable overlap between months, temporal change was significant on both PC1 ($F_{4,195} = 18.524$, $p < 0.001$) and PC2 ($F_{4,195} = 22.390$, $p < 0.001$). PC1 scores were similar and low in May and June, reflecting relatively high DO availability, increased in July then peaked in August as DO availability declined. PC2 scores were high in May, June, and September, reflecting lower temperatures and higher conductivity in these months. PC2 scores were particularly low in July, reflecting low conductivities and high temperatures (Figure 6.12(i)).

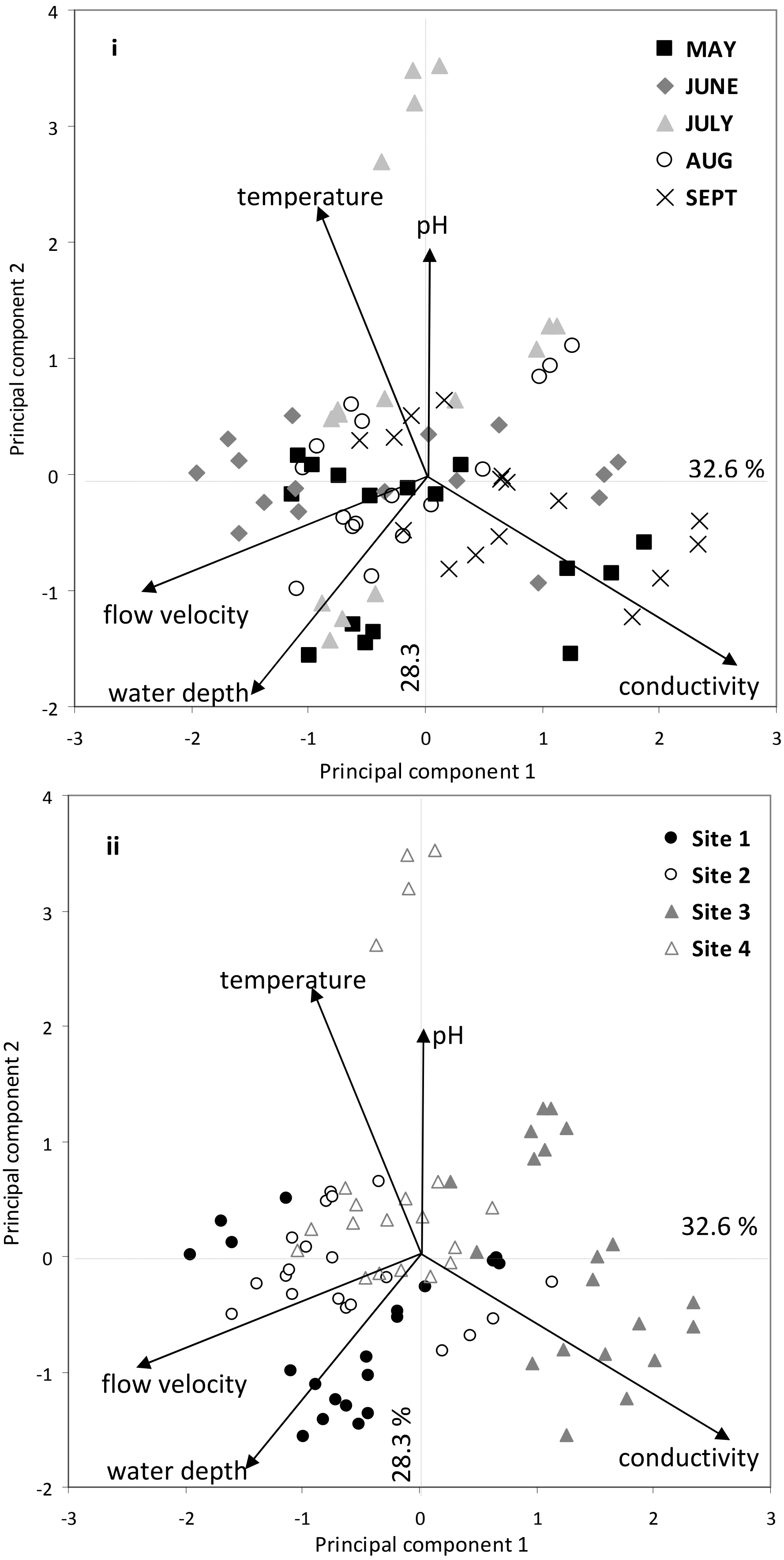


Figure 6.11: Principal components analysis of surface water data: i) temporal variability; ii) spatial variability in environmental conditions. Key: circles = West Glen; triangles = East Glen.

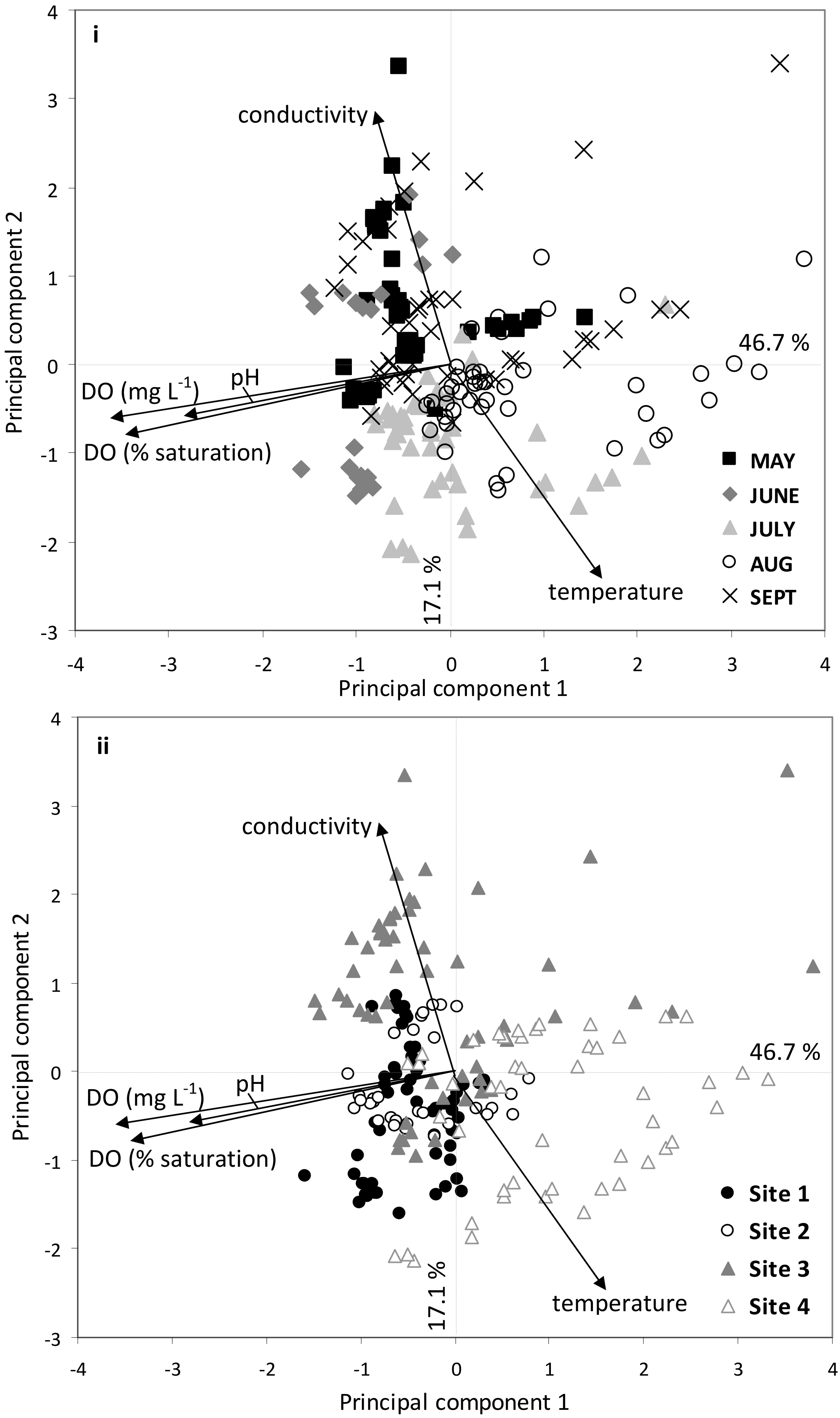


Figure 6.12: Principal components analysis of hyporheic water data: i) temporal variability; ii) spatial variability in environmental conditions. Key: circles = West Glen; triangles = East Glen.

Comparison of Figures 6.12 (i) and (ii) indicated that much environmental variation was explained by site-specific differences. PC1 scores were similar at sites 1, 2 and 3 but significantly higher at site 4 ($F_{3, 196} = 34.848$, $p < 0.001$), reflecting the low DO concentrations recorded at the latter site (Figure 6.12(ii)). PC2 scores were similar at sites 1, 2 and 4 and higher at site 3 ($F_{3, 196} = 37.026$, $p < 0.001$), reflecting high conductivity and temperature values recorded at site 3. West Glen sites 1 and 2 formed a tight, partly overlapping cluster at the centre of the ordination, whilst East Glen sites were separated on PC2, and had highly variable scores on PC1, reflecting the wide range of DO values recorded at these sites (Figure 6.12(ii)).

6.5 Spatiotemporal variability in the benthic invertebrate community

Spatial and temporal variability in the composition of the invertebrate community inhabiting the benthic sediments is examined to address two aims: firstly, to analyse changes in the abundance of taxa with the potential to increase the strength of biotic interactions (aim 1, objective 6), and secondly, to consider changes in community composition arising as a result of variation in hydrological or hydrologically-mediated (biotic and abiotic) variables (aim 2, objectives 1 and 2).

Community description

A total of 54,532 invertebrates were recorded from 80 Surber samples taken from the benthic sediments. The community comprised members of 103 taxa, including 72 identified to species level and 31 higher taxa that may have included multiple representatives.

6.5.1 Detrended correspondence analysis

DCA was conducted to investigate spatial and temporal variability in community composition (Figures 6.13 and 6.14). Axis 1 explained 18.5 % of the variation in the species data and had significant ($p < 0.05$) correlations with the abundance of just two taxa, *Oulimnius* spp. (larvae) and *G. pulex*. Axis 2 explained a further 11.8 % of the variance and had highly significant ($p < 0.01$) correlations with *P. antipodarum*, Sphaeriidae, *E. octoculata*, *Oulimnius* (larvae), *S. ignita* and *Baetis* spp. abundance.

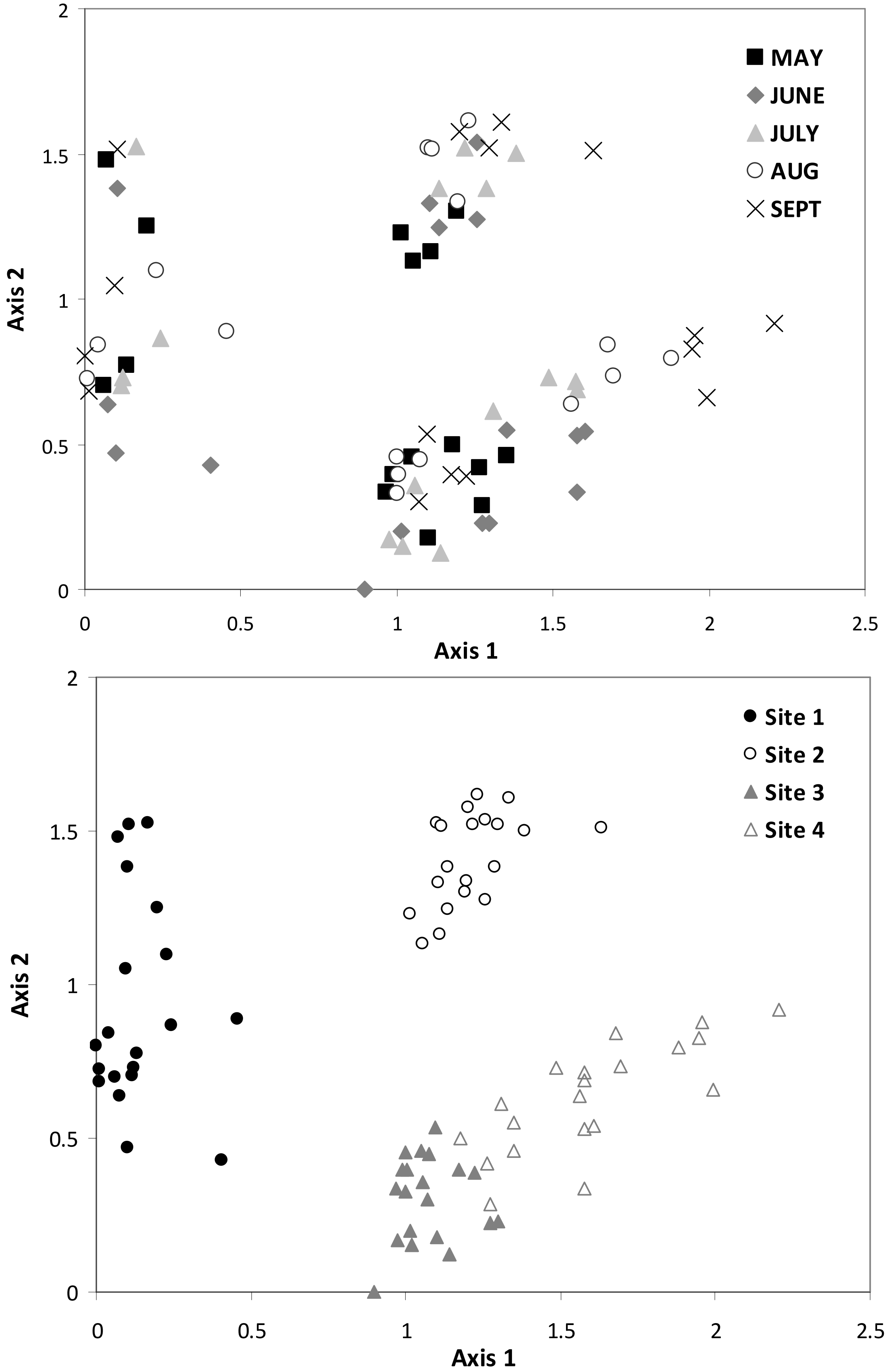


Figure 6.13: Detrended correspondence analysis sample plot of benthic community data: i) temporal variability; ii) spatial variability. Key: circles = West Glen; triangles = East Glen.

Temporal variability

Considering all sites, temporal change in community composition was demonstrably not responsible for the observed clustering, with samples from all months present in each cluster (Figure 6.13(i)). However, when site-specific differences were taken into account using RM ANOVA, temporal change was significant on both axis 1 ($F_{2.083, 31.244} = 5.652$, $p = 0.007$) and axis 2 ($F_{4, 60} = 9.345$, $p < 0.001$). Axis 1 scores were lowest in May, moderate between June and August then peaked in September; axis 2 scores tended to increased in each month, although a June dip was observed at sites 1 and 2 (Figure 6.13(i)).

Positioning of some taxa on the species plot (Figure 6.14) reflected their temporal occurrence, for example Simuliidae were particularly abundant in May and plotted in the negative quadrant of axis 1. In other cases, temporal associations were less apparent, for example the Sphaeriidae had a similar axis 1 score to the Simuliidae despite peak abundance in August. Ubiquitous taxa, (Chironomidae, Oligochaeta), plotted at the centre of the species ordination, regardless of temporal change in their occurrence (Figure 6.14).

Spatial variability

The clustering observed in the sample plots reflected spatial variability between sites, with West Glen sites 1 and 2 forming two distinct groups and East Glen sites 3 and 4 plotting with slight overlap as a single group (Figure 6.13(ii)). West Glen sites had higher axis 2 scores than East Glen sites ($F_{1, 14} = 26.531$, $p < 0.001$), whilst intermittent sites (2, 4) had higher axis 1 scores than perennial sites (1, 3; $F_{1, 14} = 17.393$, $p = 0.001$).

Spatiotemporally ubiquitous taxa plotted near the centre of species ordination (Figure 6.14), whilst the positioning of others (e.g. Simuliidae) could be linked to their temporal occurrence. Other taxa plotted according to their spatial distribution, for example Sphaeriidae occurred almost exclusively at site 1 and had a low axis 1 score despite their temporal occurrence. Accordingly, taxa such as *Oulimnius* larvae, which were largely found at sites 2 and 4, scored highly on axis 1 (Figure 6.14).

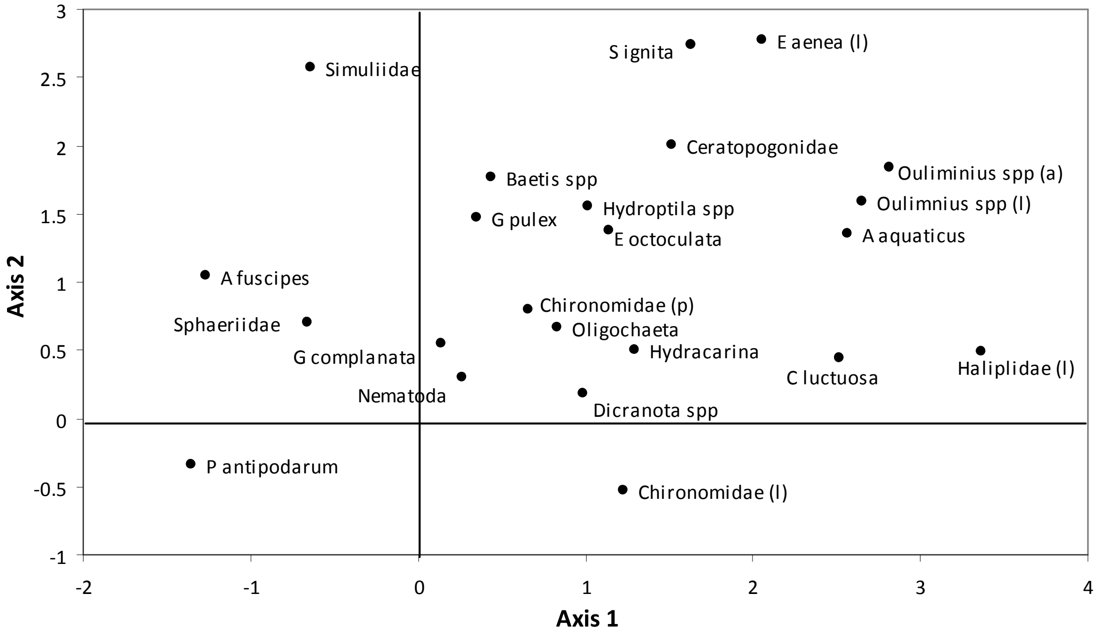


Figure 6.14: Detrended correspondence analysis species plot of benthic community data. All common invertebrate taxa (those accounting for >0.5% of the total community) are indicated.

6.5.2 Community metrics

Four metrics were calculated to summarise the benthic invertebrate community and allow characterisation of temporal change in community composition: total invertebrate abundance, taxon richness, the Berger-Parker dominance index and Simpson's Diversity Index (see section 4.9.1).

Total invertebrate abundance (TIA)

TIA varied between 84 individuals 0.1 m^{-2} at site 3 in June up to 2849 0.1 m^{-2} (mainly due to high chironomid densities) at site 4 in May. Spatial variation between sites was not significant (Table 6.9). Considering all sites, TIA was particularly high in May and lowest in August and September ($F_{4, 60}$ = 6.237, p <0.001; Table 6.8; Figure 6.15(i)). The interaction between TIA and site was significant ($F_{12, 48}$ = 4.195, p <0.001), and temporal change was only significant at site 4. Here abundance was highest in May and also high in July, then declined by 77 % between July and August and dropped further in September ($F_{1.456, 4.368}$ = 15.293, p = 0.012).

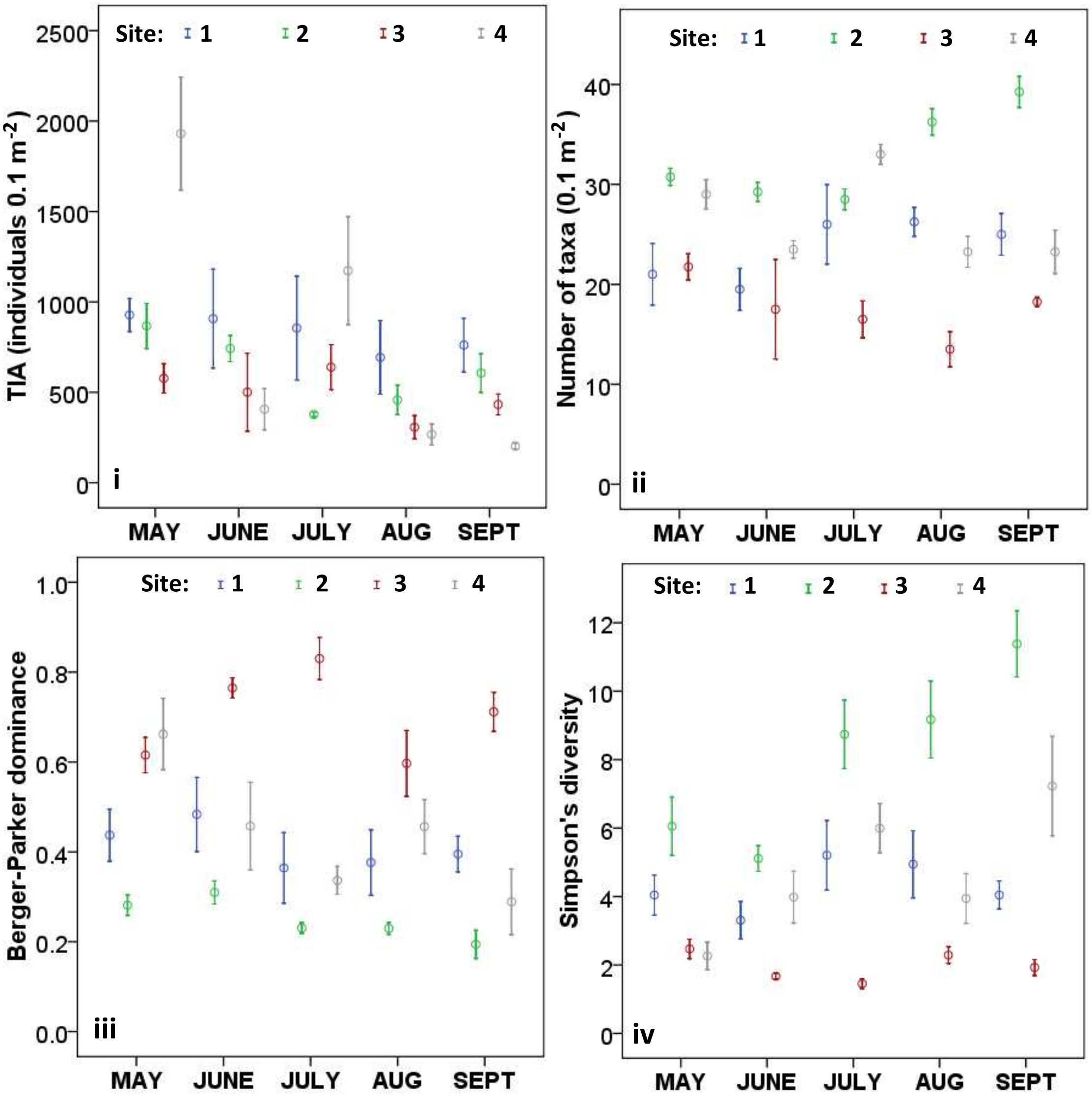


Figure 6.15: Mean ± 1 SE benthic community metrics: i) total abundance (TIA; individuals 0.1m^{-2}); ii) number of taxa (taxa 0.1m^{-2}); iii) Berger-Parker dominance; iv) Simpson's diversity.

Taxon richness

The number of taxa per sample (0.1 m^{-2}) varied between 5 taxa at site 3 in June to 42 taxa recorded at site 2 in September. Spatial differences in mean richness were site-specific, being highest at site 2 and particularly low at site 3 ($F_{3, 12}$ = 21.090, p <0.001; Table 6.9). Overall, mean richness was stable between months ($F_{4, 60}$ = 1.651, p = 0.173; Table 6.8; Figure 6.15(ii)); however the interaction between taxa and site was significant ($F_{12, 48}$ = 3.369, p = 0.001) and taxon richness varied significantly at two sites. At site 2, taxon richness was stable between May and July then increased to a September peak ($F_{2.188, 6.563}$ = 13.684, p = 0.004) due to increases in Gastropoda, Turbellaria and Coleoptera taxa. At site 4, the number of taxa was highest in July, with *Agabus didymus* and *Helophorus brevipalpis* (Coleoptera) occurring exclusively in this month; richness then declined by 29.5 % (10 taxa) in August and stayed low in September ($F_{1.774, 5.322}$ = 7.659, p = 0.029; Figure 6.15(ii)).

Berger-Parker dominance

Berger-Parker dominance varied between 0.15 in a site 4 September sample up to 0.94 in a chironomid-dominated site 3 sample taken in July. Spatial differences were site-specific, with dominance being particularly low at site 2, moderate at sites 1 and 4, and very high at site 3 ($F_{3, 12}$ = 57.793, p <0.001; Table 6.9). Overall, dominance was highest in May and June then declined in each subsequent month, but temporal change was not significant ($F_{2.186, 32.785}$ = 2.209, p = 0.122; Table 6.8; Figure 6.15(iii)). The interaction between dominance and site was, however, significant ($F_{12, 48}$ = 2.709, p = 0.007), with the overall temporal pattern being observed only at site 2 ($F_{2.017, 6.051}$ = 5.876, p = 0.038). Contrasting patterns were observed at other sites, for example dominance was stable at site 1, and was particularly variable at site 4, ranging from 0.66 ± 0.08 in May down to 0.29 ± 0.07 in September (Figure 6.15(iii)).

Table 6.8: Temporal change in benthic community metrics in the River Glen, May to September 2008

Community metric	**May**	**June**	**July**	**August**	**Sept**	**Temporal change**
Total invertebrate abundance (ind. $0.1m^{-2}$)	1075 ± 154	639 ± 98	761 ± 122	431 ± 68	500 ± 69	**
Number of taxa (taxa $0.1m^{-2}$)	25.6 ± 1.4	22.4 ± 1.7	26.0 ± 1.9	24.8 ± 2.2	26.4 ± 2.2	ns
Berger-Parker dominance	0.50 ± 0.05	0.50 ± 0.05	0.44 ± 0.06	0.41 ± 0.04	0.40 ± 0.05	ns
Simpson's diversity	3.7 ± 0.5	3.5 ± 0.4	5.3 ± 0.8	5.1 ± 0.8	6.1 ± 1.0	**

All values presented as mean ± 1 SE. Temporal change analysed using one-way RM ANOVA, with * and ** indicating overall significance levels of p <0.01 and n/s indicating p >0.05. Key – ind. = individuals.

Table 6.9: Spatial differences in benthic community metrics at River Glen sites 1-4

Community metric	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Spatial change**
Total invertebrate abundance (ind. $0.1m^{-2}$)	829 ± 87	610 ± 54	491 ± 56	796 ± 172	ns
Number of taxa (taxa $0.1m^{-2}$)	23.6	32.8	17.5	26.4	**
Berger-Parker dominance	0.41 ± 0.03	0.25 ± 0.01	0.70 ± 0.03	0.44 ± 0.04	**
Simpson's diversity	4.3 ± 0.34	8.1 ± 0.63	2.0 ± 0.12	4.7 ± 0.53	**

All values presented as mean ± 1 SE. Spatial change analysed using two-way RM ANOVA, with * and ** indicating overall significance levels of p <0.01 and n/s indicating p >0.05. Key – ind. = individuals.

Simpson's diversity

Diversity was very low (1.12) in the chironomid-dominated sample taken at site 3 in July, whilst the highest value (13.2) was recorded at site 2 in September. Spatial variability was the opposite of that reported for dominance, being very low at site 3 and particularly high at site 2 ($F_{3, 12} = 41.064$, $p < 0.001$; Table 6.9)). Temporally, diversity was lowest in May and June and highest in September; temporal change was significant ($F_{2.390, 35.850} = 6.386$, $p = 0.003$; Table 6.8; Figure 6.15(iv)), as was the interaction with site ($F_{6.511, 26.042} = 3.975$, $p = 0.005$). Temporal change was only significant at site 2, with diversity being low in June then increasing monthly to a September peak ($F_{1.616, 4.849} = 11.872$, $p = 0.015$; Figure 6.15(iv)).

6.5.3 Abundance of common taxa

Taxon abundances were examined firstly, to determine changes in the occurrence of dominant species with the potential to increase the strength of biotic interactions, and secondly, to consider changes in abundance resulting from variation in hydrological, environmental or biotic variables. Thirteen taxa each accounted for >1 % TIA and these taxa cumulatively comprised 88.7 % of the benthic community (Table 6.10). In addition, three regionally or nationally notable species were present at very low abundance: *Mesovelia furcata* (Hemiptera), the genus *Riolus* (Coleoptera) and *Gyrinus urinator* (Coleoptera) (Chadd and Extence, 2004).

Table 6.10: Occurrence of common benthic invertebrates (>1 % total invertebrate abundance) in the River Glen

Taxon	Total no. of individuals	% of community	Cumulative % of community	Present in *x* % of samples
CHIRONOMIDAE (l)	19532	35.8	35.8	100
OLIGOCHAETA	8637	15.8	51.7	100
Baetis spp.	4381	8.0	59.7	85
Potamopyrgus antipodarum	3878	7.1	66.8	40
SPHAERIIDAE	2935	5.4	72.1	81.3
Simuliidae (l)	1993	3.7	75.8	55
Oulimnius spp. (l)	1347	2.5	78.3	73.8
Gammarus pulex	1086	2.0	80.3	92.5
HYDRACARINA	1004	1.8	82.1	90
Erpobdella octoculata	986	1.8	83.9	97.5
Caenis luctuosa group	944	1.7	85.7	60
Hydroptila spp. (l)	875	1.6	87.2	63.8
Serratella ignita	769	1.4	88.7	31.3
Total	48367	88.7	88.7	

Chironomidae larvae

Larvae of the family Chironomidae (Diptera) experience seasonal changes in abundance due to adult emergence, but were by far the most abundant benthic taxon, accounting for 35.8 % TIA and occurring in all samples, and are therefore considered briefly here. Spatially, chironomids were more abundant at both East Glen sites compared with the West Glen ($F_{1, 14}$ = 46.517, p <0.001; Table 6.12). Temporally, chironomid abundance was highest in May and lowest in August and September ($F_{2.282, 34.232}$ = 9.008, p <0.001; Table 6.11); the interaction with site was significant ($F_{3, 12}$ = 16.568, p <0.001), but minor variations from the overall pattern of change were not significant at individual sites.

Table 6.11: Temporal change in the abundance of common benthic taxa in the River Glen, May to September 2008.

	Mean ± 1 SE abundance (individuals 0.1m^{-2})					Temporal change
	May	**June**	**July**	**August**	**Sept**	
CHIRONOMIDAE (l)	520 ± 154	232 ± 56	287 ± 66	94 ± 21	88 ± 34	**
OLIGOCHAETA	132 ± 23	125 ± 31	110 ± 26	89 ± 14	84 ± 19	ns
P. antipodarum	52 ± 33	65 ± 38	56 ± 34	23 ± 17	46 ± 34	ns
SPHAERIIDAE	18 ± 11	30 ± 17	33 ± 18	62 ± 44	39 ± 20	ns
SIMULIIDAE (l)	58 ± 35	13 ± 9	13 ± 6	20 ± 11	21 ± 17	ns
Gammarus pulex	9.6 ± 3.4	8.9 ± 2.0	24.8 ± 9.0	9.3 ± 2.9	15.3 ± 4.3	ns
HYDRACARINA	9.8 ± 2.1	5.8 ± 2.6	39 ± 24	2.9 ± 0.7	5.3 ± 1.6	ns
Erpobdella octoculata	15 ± 3.6	12 ± 2.7	8.4 ± 1.3	12 ± 2.2	14 ± 4.2	ns

All values presented as mean ± 1 SE. Temporal change analysed using one-way RM ANOVA; * indicates p <0.05, ** indicates p <0.01

Table 6.12: Spatial differences in the abundance of common benthic taxa at River Glen sites 1-4.

	Mean ± 1 SE abundance (individuals 0.1m^{-2})				Spatial change
	Site 1	**Site 2**	**Site 3**	**Site 4**	
CHIRONOMIDAE (l)	111 ± 30	112 ± 21	355 ± 51	398 ± 135	**
OLIGOCHAETA	133 ± 29	104 ± 18	69 ± 10	126 ± 21	ns
P. antipodarum	1189 ± 44	0	4.9 ± 1.2	0	*
SPHAERIIDAE	133 ± 36	6.8 ± 1.2	3.3 ± 0.8	3.1 ± 1.0	*
SIMULIIDAE (l)	81 ± 30	17 ± 8	0	0.9 ± 0.5	ns
Gammarus pulex	23.9 ± 5.0	14.0 ± 3.0	1.9 ± 0.4	14.6 ± 6.2	**
HYDRACARINA	6.1 ± 2.1	5.8 ± 1.3	4.1 ± 1.0	34.4 ± 19.5	**
Erpobdella octoculata	7.3 ± 1.4	26 ± 3.2	11.5 ± 1.5	4.6 ± 0.8	**

All values presented as mean ± 1 SE. Spatial change analysed using one-way RM ANOVA; * indicates p <0.05, ** indicates p <0.01, ns indicates p >0.05

Oligochaeta

Species of the Oligochaeta comprised 15.8 % TIA and were present in all samples at mean densities of 108 ± 11 0.1 m^{-2}, occurring at comparable abundance at all sites (Table 6.12). Oligochaete abundance peaked in May then declined in each subsequent month, but temporal change was not significant ($F_{4, 60} = 0.826$, $p = 0.514$; Table 6.11); neither was there a significant interaction with site.

Potamopyrgus antipodarum

P. antipodarum (Gastropoda) accounted for 7.1 % TIA but was patchily distributed, occurring in 40 % of samples at densities of ≤560 0.1 m^{-2}. *P. antipodarum* was more abundant at perennial than intermittent sites ($F_{1, 14} = 7.169$, $p = 0.018$), being particularly common at site 1 and absent from sites 2 and 4 (Table 6.12). Abundance was low in August and twice as high in other months but temporal change was not significant ($F_{1.758, 26.364} = 1.082$, $p = 0.346$; Table 6.11).

Sphaeriidae

Species of the family Sphaeriidae (Bivalvia) comprised 5.4 % TIA and were widespread, occurring in 81.3 % of samples at mean densities of 36 ± 11 0.1 m^{-2}, but being particularly abundant at site 1 ($F_{3, 12} = 7.736$, $p = 0.004$; Table 6.12). Abundances were lowest in May then increased gradually to a peak in August; however, neither temporal change nor interactions with spatial parameters were significant (Table 6.11).

Gammarus pulex

The amphipod *G. pulex* comprised 2 % TIA and was widespread, occurring in 92.5 % of samples at densities of ≤120 0.1 m^{-2}. Mean abundance of *G. pulex* was comparable at sites 1, 2 and 4 and low at site 3 ($F_{3, 12} = 8.504$, $p = 0.003$; Table 6.12). Considering all sites, *G. pulex* abundance was similar and low in May, June and August and highest in July, however, these changes were not significant ($F_{2.002, 30.027} = 2.354$, $p = 0.112$; Table 6.11). The interaction with site was significant ($F_{12, 48} = 2.811$, $p = 0.005$), with the overall pattern representing conditions only at site 1. At site 2, *G. pulex* abundance increased each month to a September peak, at site 3, abundance remained low in all months, and at site 4, abundance was 4x higher in July than in other months then fell sharply in August and remained low in September; temporal change was not significant at individual sites.

Hydracarina

The Hydracarina (Acari) accounted for 1.8 % of all invertebrates and was widespread, occurring in 90 % of samples at mean densities of 12.6 ± 5.0 0.1 m^{-2}. Abundance varied between sites, the taxon occurring at comparable low densities at sites 1-3 but being far more abundant at site 4 ($F_{3, 12} = 7.747$, $p = 0.004$; Table 6.12). Hydracarina were considerably more abundant in July than in any other month, but this temporal variability was not significant ($F_{1.518, 22.772} = 2.591$, $p = 0.108$; Table 6.11). The interaction between abundance and site was significant ($F_{5.747, 22.988} = 5.300$, $p = 0.002$), and the July peak occurred only at site 4, where it was followed by a reduction in abundance of >99 % in August ($F_{1.164, 1.448} = 6.937$, $p = 0.065$).

Erpobdella octoculata

E. octoculata (Hirudinea) accounted for 1.8 % TIA and was very widespread, occurring in 97.5 % of samples at densities of ≤60 0.1 m^{-2}. The taxon occurred at comparable densities at sites 1, 3 and 4, with significant spatial variability reflecting high abundances at site 2 ($F_{3, 12} = 12.278$, $p = 0.001$; Table 6.12). *E. octoculata* declined in abundance between May and July then became increasingly common in subsequent months, but temporal change was not significant ($F_{4, 60} = 1.007$, $p = 0.411$; Table 6.11). The interaction with site was significant ($F_{12, 48} = 4.553$, $p < 0.001$), with the overall pattern being observed and significant at site 2 ($F_{2.180, 6.539} = 7.940$, $p = 0.017$); contrasting patterns were recorded elsewhere, for example at site 4 abundance increased to a July peak then declined in both August and September, but temporal change was not significant at any other site.

Other common taxa

Baetis spp., Simuliidae, *Caenis luctuosa* group, *Hydroptila* spp., *Ouliminius* spp. and *Serratella ignita* were all common members of the benthic community (Table 6.10). However, larvae and pupae of the Insecta are commonly absent from the aquatic environment in summer following emergence of terrestrial adult life stages (Williams and Feltmate, 1992; Elliott, 2006); any attempt to relate their abundance to hydrological conditions is liable to be confounded by seasonal changes. They are therefore not appropriate for further consideration in the current investigation, except to note that Simuliidae larvae abundance declined considerably between May (58 ± 35 0.1 m^{-2}) and June (13 ± 9 0.1 m^{-2}; Table 6.11).

6.6 Spatiotemporal variability in the hyporheic invertebrate community

The composition of the community resident in the hyporheic zone is examined using the same methods as applied to benthic community data (aim 2, objective 1). Particular attention is paid to temporal change in the hyporheic occurrence of predominantly benthic taxa (objective 2).

Community description

A total of 5165 invertebrates were recorded in 232 samples pumped from the hyporheic zone. Of these, 2169 were from a depth of 10 cm, 1326 from 20 cm and 1670 from 30cm. At least 63 taxa were present, including 37 identified to species level and 26 higher taxa that probably contained multiple representatives.

6.6.1 Detrended correspondence analysis

Preliminary DCA indicated that axes scores (and therefore community composition) were comparable at the three hyporheic depths ($p > 0.5$) and all were therefore combined in the subsequent analyses. DCA was used to examine both spatial and temporal variability in community composition (Figure 6.16). Axis 1 explained 10.8 % of the species variation and was significantly correlated with several common taxa, in particular Simuliidae larvae and *Caenis luctuosa* group ($p < 0.001$). Axis 2, which explained an additional 9.8 % of the variation, had highly significant ($p < 0.001$) correlations with taxa including Simuliidae larvae and Sphaeriidae.

Temporal variability

Samples from all months formed an overlapping cluster towards the centre of the ordination (Figure 6.16(i)). May samples formed a relatively tight group that overlapped with all other months, whilst September samples spanned Axis 1 and June samples were particularly variable on axis 2. Despite this variability, temporal change was significant on both axes. Axis 1 scores declined between May and July then increased to a peak in September ($F_{2.802,\ 109.274} = 7.490$, $p < 0.001$), whilst axis 2

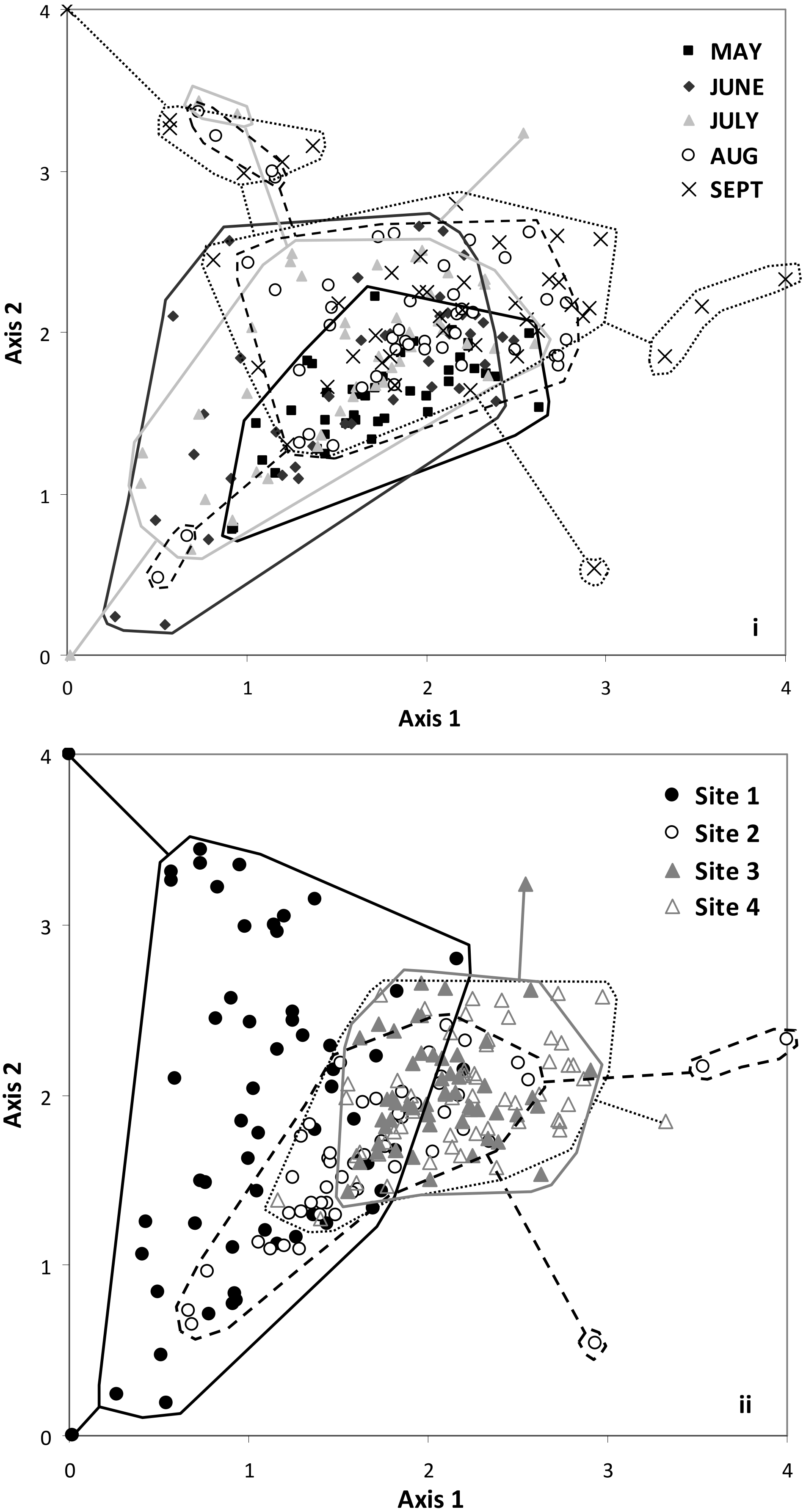


Figure 6.16: Detrended correspondence analysis sample plot of the hyporheic community: i) temporal variability; ii) spatial variability. Key: circles = West Glen; triangles = East Glen.

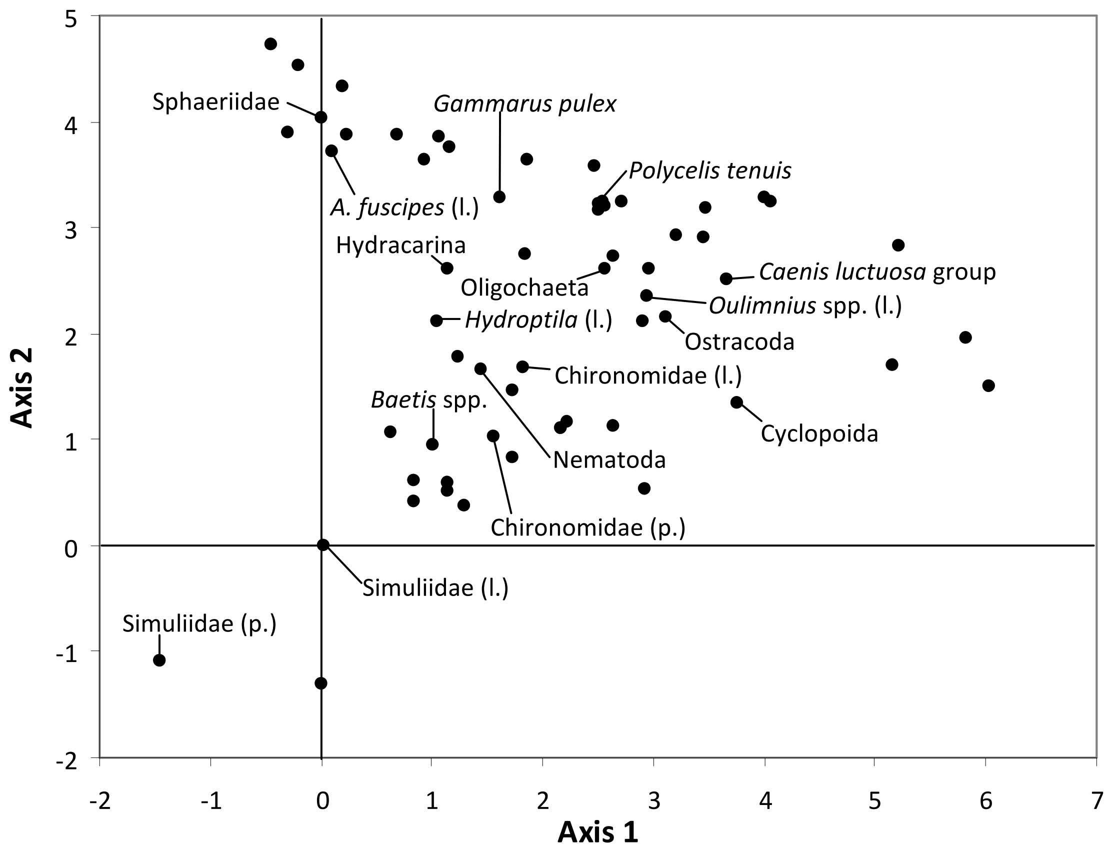


Figure 6.17: Detrended correspondence analysis species plot of hyporheic community data. All common invertebrate taxa (>0.5 % of the total community) are indicated.

scores were lowest in May then increased each month to a peak in September ($F_{2.997, 116.895} = 17.541$, $p < 0.001$; Figure 6.16(i)). Significant interactions between site and axis scores indicated that patterns of temporal change were site-specific.

Positioning of some taxa on the species plot (Figure 6.17) could be attributed to their temporal occurrence, for example the Sphaeriidae were most abundant in August and September and had high axis 2 scores. Similarly, abundance of Simuliidae larvae peaked in June and was very low in September, as reflected by low axis 2 scores. Ubiquitous taxa such as the Chironomidae plotted towards the centre of the species plot despite significant temporal changes in their abundance.

Spatial variability

Whilst the tight clustering of samples into site-specific groups observed for the benthic community was absent, hyporheic communities of certain sites could be distinguished (Figure 6.16(ii)). In particular, site 1 samples spanned the length of axis

2, indicating community variability, but had low axis 1 scores. In contrast, site 3 and 4 samples formed a relatively tight cluster at the centre of the ordination, indicating community homogeneity; these samples had higher axis 1 scores than West Glen sites. Between-site variability was significant on axis 1 ($F_{3, 36}$ = 100.105, p <0.001) whilst axis 2 scores were comparable at all sites (Figure 6.16(ii)).

The association of certain taxa with specific sites was reflected by their positioning in the species plot (Figure 6.17). *Caenis luctuosa* group, for example, occurred at all sites except site 1 and scored highly on axis 1, whilst most Simuliidae larvae were recorded at site 1 and had a much lower axis 1 score.

6.6.2 Community metrics

Total invertebrate abundance (TIA)

TIA peaked at 136 6 L^{-1} in a 10 cm depth sample taken at site 4 in May, whilst no invertebrates were present in six samples. Abundance was comparable at 10 cm (27 ± 3.3 6 L^{-1}) and 30 cm (23 ± 3.2 6 L^{-1}) but lower at 20 cm (16 ± 2.4 6 L^{-1}; $F_{2, 41}$ = 5.158, p = 0.010). The interaction with hyporheic depth was not significant ($F_{8, 164}$ = 0.585, p = 0.790) and all depths were therefore pooled in subsequent analyses. Spatially, TIA was comparable at sites 1, 2 and 3 and higher at site 4 ($F_{3, 40}$ = 8.224, p <0.001; Table 6.14). TIA was highest in May, moderate between June and August and lowest in September ($F_{4, 172}$ = 9.927, p <0.001; Table 6.13; Figure 6.18(i)). The interaction between TIA and site was significant ($F_{12, 160}$ = 3.167, p <0.001) and patterns of temporal change were site-specific. At site 1, TIA was highest in June but temporal change was not significant; at site 2, the overall pattern was observed ($F_{4, 28}$ = 6.224, p = 0.001); the same pattern was seen at site 3 but was not significant; and at site 4, there was a pronounced peak in abundance in May (75 ± 12 6 L^{-1}) and abundance was also high in July ($F_{4, 44}$ = 10.864, p <0.001).

Taxon richness

Taxon richness peaked at 16 taxa 6 L^{-1} in a sample taken at site 2 in August, whilst no invertebrates were recorded in six samples. The number of taxa was higher at 10 cm (6.0 ± 0.3 taxa 6 L^{-1}) than at both 20 cm (3.9 ± 0.3 taxa 6 L^{-1}) and 30 cm (4.3 ± 0.3 taxa 6 L^{-1}; $F_{2, 41} = 7.609$, $p = 0.002$), but the interaction with depth was not significant ($F_{8, 164} = 0.937$, $p = 0.487$). Taxon richness was particularly low at site 3 and was highest at site 4 ($F_{3, 40} = 4.604$, $p = 0.007$; Table 6.14). Temporally, mean taxon richness was high in May, moderate from June to August and low in September ($F_{4, 172} = 3.016$, $p = 0.024$; Table 6.13; Figure 6.18(ii)), and the interaction between taxa and site was not significant ($F_{12, 160} = 1.384$, $p = 0.178$).

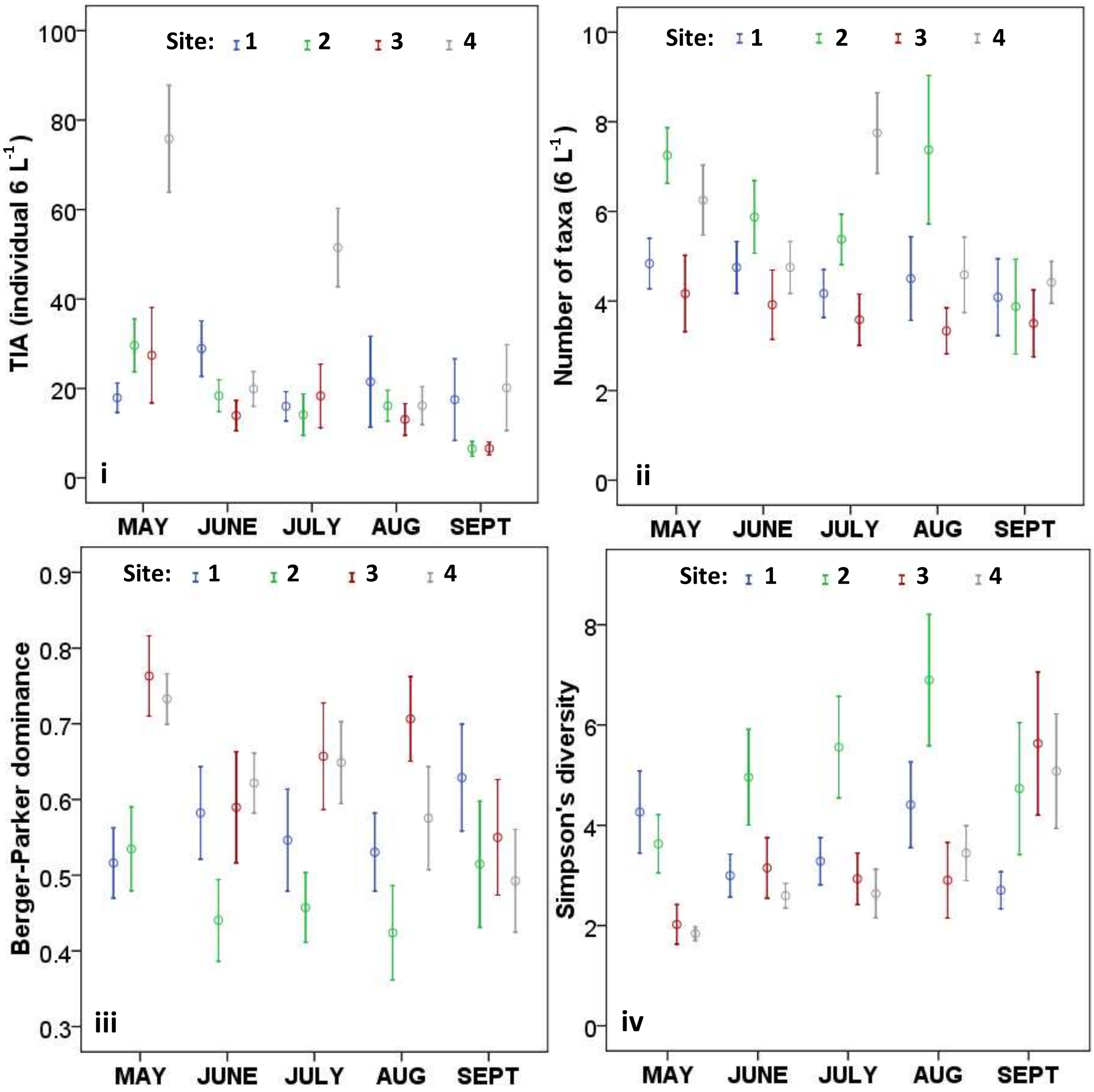


Figure 6.18: Mean ± 1 SE temporal change in hyporheic community metrics: i) total invertebrate abundance (TIA; individuals 6 L^{-1}); ii) number of taxa (taxa 6 L^{-1}); iii) Berger-Parker dominance; iv) Simpson's diversity

Table 6.13: Temporal change in hyporheic community metrics in the River Glen, May to September 2008

Community metric	May	June	July	August	Sept	Temporal change
Total abundance (individuals 6 L^{-1})	38.4 ± 5.6	20.5 ± 2.4	26.0 ± 4.0	16.8 ± 3.1	13.3 ± 3.7	**
Taxon richness (taxa 6 L^{-1})	5.5 ± 0.4	4.7 ± 0.3	5.2 ± 0.4	4.7 ± 0.5	4.0 ± 0.4	*
Berger-Parker dominance	0.64 ± 0.03	0.58 ± 0.03	0.58 ± 0.03	0.58 ± 0.03	0.55 ± 0.04	ns
Simpson's diversity	2.9 ± 0.3	3.3 ± 0.3	3.6 ± 0.4	4.1 ± 0.5	4.7 ± 0.6	*

Values presented as mean ± 1SE of all samples. Temporal change analysed using one-way RM ANOVA; * indicates p <0.05, ** indicates p <0.01, ns indicates p >0.05.

Table 6.14: Spatial differences in hyporheic community metrics at River Glen sites 1-4

Community metric	Site 1	Site 2	Site 3	Site 4	Spatial change
Total abundance (individuals 6 L^{-1})	20.4 ± 3.1	15.2 ± 1.7	15.9 ± 2.8	36.7 ± 4.7	**
Taxon richness (taxa 6 L^{-1})	4.5 ± 0.3	5.4 ± 0.4	3.7 ± 0.3	5.6 ± 0.4	**
Berger-Parker dominance	0.56 ± 0.03	0.47 ± 0.03	0.66 ± 0.03	0.61 ± 0.03	**
Simpson's diversity	3.5 ± 0.3	5.3 ± 0.5	3.3 ± 0.4	3.1 ± 0.3	**

Values presented as mean ± 1SE of all samples. Spatial change analysed using two-way RM ANOVA; ** indicates p <0.01

Berger-Parker dominance

Dominance ranged from 1 (complete dominance) in several samples containing a single taxon, down to 0.14 in a sample taken from site 2 in September, which contained seven taxa at low abundance. Dominance values were relatively low at a depth of 10 cm (0.52 ± 0.02) and higher at 20 cm (0.61 ± 0.03) and 30 cm (0.60 ± 0.02; $F_{2, 40}$ = 4.460, p = 0.018). Site-specific differences were significant ($F_{3, 39}$ = 7.049, p = 0.001), with mean dominance being lowest at site 2 and highest at site 3 (Table 6.14). Dominance was highest in May, stable and moderate between June and August, and lower in September (Table 6.13; Figure 6.18(iii)); temporal change was not significant.

Simpson's diversity

The lowest diversity value possible (1) was recorded in all samples containing a single taxon, whilst diversity peaked at 16.5 in a site 3 sample taken in September; this sample comprised nine taxa at low abundance. Diversity peaked at 10 cm but was comparable at all hyporheic depths ($F_{2, 40}$ = 2.117, p = 0.134). Spatially, diversity was comparable at sites 1, 3 and 4 and significantly higher at site 2 ($F_{3, 39} = 8.075$, $p < 0.001$; Table 6.14). Overall, diversity increased gradually from May to a peak in September ($F_{2.828, 118.774} = 2.776$, $p = 0.047$; Figure 6.18(iv); Table 6.14). However, the interaction between site and diversity was significant ($F_{8.505, 110.569} = 2.009$, $p = 0.048$), with the overall pattern being observed at sites 3 and 4; temporal change was not significant at sites 1 or 2 (Figure 6.18(iv)).

6.6.3 Abundance of common taxa

The abundance of taxa common in the hyporheic zone was examined, primarily to identify any changes in the use of the hyporheic zone by benthic invertebrates. Eleven taxa each comprised >1 % TIA, and together these taxa accounted for 90.8 % of the hyporheos (Table 6.15). In addition to these common taxa, two regionally-notable species of conservation interest were present at very low abundance: *Mesovelia furcata* (also present in the benthic sediments) and the groundwater specialist *Niphargus aquilex* (Amphipoda) (Chadd and Extence, 2004).

Table 6.15: Occurrence of common invertebrates (>1 % total invertebrate abundance) in the hyporheic zone of the River Glen

Taxon	**Total no. of individuals**	**% of community**	**Cumulative % of community**	**Present in *x* % of samples**
CHIRONOMIDAE (l)	2133	41.3	41.3	85.3
OLIGOCHAETA	849	16.4	57.7	60.0
SIMULIIDAE (l)	440	8.5	66.3	31.5
SPHAERIIDAE	323	6.3	72.5	19.0
Baetis spp.	268	5.2	77.7	33.6
NEMATODA (meiofauna)	186	3.6	81.3	31.9
CYCLOPOIDA (meiofauna)	140	2.7	84.0	21.6
CERATOPOGONIDAE (pupae)	122	2.4	86.4	1.7
HYDRACARINA	82	1.6	88.0	21.1
Caenis luctuosa group	79	1.5	89.4	18.9
Gammarus pulex	67	1.3	90.8	14.7
Total	4689	90.8		

Chironomidae larvae

Chironomid larvae dominated the hyporheic community, accounting for 41.3 % TIA and occurring in 85.3 % of samples at mean densities of 9.2 ± 1.1 6 L^{-1}. Vertically, the taxon was similarly abundant at depths of 10 cm and 30 cm, but less common at 20 cm ($F_{2, 41} = 4.938$, $p = 0.012$). Spatially, chironomids were less numerous at West Glen than East Glen sites ($F_{1, 42} = 17.695$, $p < 0.001$), with particularly high abundance recorded at site 4 (Table 6.17). Chironomid abundance was particularly high in May, declined sharply in June and continued to fall in each subsequent month ($F_{2.469, 106.159}$ = 30.402, p <0.001; Table 6.16); the interaction with site was significant ($F_{9.277, 123.688}$ = 4.734, p <0.001). At site 1, May abundances were only slightly higher than in subsequent months; at sites 2 and 3, abundance rose between July and August before falling to a five-month low in September; and at site 4, chironomid abundance was particularly high in May and very low in August and September; temporal change was significant at all individual sites ($p \leq 0.047$).

Oligochaeta

Species of oligochaete comprised 16.4 % TIA and occurred in 60 % of samples at mean densities of 3.7 ± 0.8 6 L^{-1}; the taxon declined in abundance with increasing depth but this was not significant. Oligochaetes were more common on the East Glen than the West Glen ($F_{1, 42}$ = 4.338, p = 0.043), with numbers being highest at site 4 (Table 6.17). Overall, oligochaete abundance was lowest in May and June then peaked in July, but temporal change was not significant ($F_{2.321, 99.797}$ = 1.802, p = 0.164; Table 6.16); neither were there significant interactions with spatial parameters.

Simuliidae larvae, *Baetis* spp., Ceratopogonidae pupae and *Caenis luctuosa* group

These insect taxa were all common in the hyporheic zone, however, due to the confounding factor of seasonal adult emergence, patterns of change are not considered in detail. It is of interest to note, however, that Simuliidae larvae were particularly abundant in the hyporheic zone at sites 1 and 2 in June ($F_{1.764, 75.838}$ = 5.031, p = 0.012; Tables 6.16 and 6.17).

Sphaeriidae

The family Sphaeriidae (Mollusca: Bivalvia) comprised 6.3 % of the hyporheos and were patchily distributed, being present in 19 % of samples at densities of ≤96 6 L^{-1}; densities were comparable at all hyporheic depths. Spatial variation in abundance was significant, with sphaeriids occurring at mean densities of 5.1 ± 1.8 6 L^{-1} at site 1 and being virtually absent from all other sites ($F_{3, 40}$ = 10.805, p <0.001; Table 6.17).

Considering all sites, sphaeriid abundance was lowest in May and peaked in August but temporal change was not significant overall ($F_{2.340, 100.602}$ = 2.166, p = 0.111; Table 6.16) or at site 1.

Hydracarina

The Hydracarina accounted for 1.6 % TIA, equating to 82 individuals. The taxon occurred in 21.1 % of samples at densities of ≤5 6 L^{-1}; abundances were comparably low at all hyporheic depths, all sites and all months (Tables 6.16 and 6.17).

Table 6.16: Temporal change in the abundance of common taxa in the hyporheic zone of the River Glen, May to September 2008.

	Mean ± 1 SE abundance (individuals 6 L^{-1})					**Temporal change***
	May	**June**	**July**	**August**	**Sept**	
CHIRONOMIDAE (l)	25.9 ± 4.4	8.5 ± 1.1	7.2 ± 1.4	4.1 ± 0.9	1.6 ± 0.2	**
OLIGOCHAETA	2.0 ± 0.6	1.9 ± 0.5	5.7 ± 2.7	3.4 ± 0.8	5.0 ± 2.6	ns
SIMULIIDAE (l)	2.3 ± 0.7	4.5 ± 1.6	2.3 ± 0.8	0.6 ± 0.3	0.06 ± 0.04	*
SPHAERIIDAE	0.1 ± 0.07	1.0 ± 0.5	0.8 ± 0.3	3.1 ± 2.1	1.8 ± 1.0	ns
HYDRACARINA	0.2 ± 0.09	0.3 ± 0.1	0.5 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	ns
Gammarus pulex	0.2 ± 0.08	0.3 ± 0.07	0.8 ± 0.4	0.1 ± 0.07	0.08 ± 0.04	ns

*Analysis of data from all sites using one-way RM ANOVA; * indicates p ≤0.05, ** indicates p ≤0.01, ns indicates p >0.05

Table 6.17: Spatial differences in the abundance of common taxa in the hyporheic zone of the River Glen sites 1-4.

	Mean ± 1 SE abundance (individuals 6 L^{-1})				**Spatial change**
	Site 1	**Site 2**	**Site 3**	**Site 4**	
CHIRONOMIDAE (l)	4.3 ± 0.6	5.0 ± 1.0	9.5 ± 2.0	17.4 ± 3.3	**
OLIGOCHAETA	1.7 ± 0.6	1.7 ± 0.3	2.9 ± 1.4	8.0 ± 2.6	*
SIMULIIDAE (l)	5.1 ± 1.2	1.7 ± 0.4	0	1.8 ± 0.4	**
SPHAERIIDAE	5.1 ± 1.8	0.04 ± 0.03	0.2 ± 0.09	0.08 ± 0.04	**
HYDRACARINA	0.53 ± 0.14	0.35 ± 0.10	0.13 ± 0.06	0.40 ± 0.11	ns
Gammarus pulex	0.22 ± 0.06	0.08 ± 0.04	0.07 ± 0.03	0.08 ± 0.36	**

*Analysis of data from all sampling dates using two-way RM ANOVA; * indicates p ≤0.05, ** indicates p ≤ 0.01, ns indicates p >0.05

Gammarus pulex

The shrimp *Gammarus pulex* comprised 1.3 % of the hyporheos, with just 67 individuals being recorded from 14.7 % of samples. Densities peaked at 20 6 L^{-1}, however most samples contained only single specimens. Mean densities peaked at 10 cm then declined moderately with increasing depth. Inter-site variability was significant, with more individuals occurring at site 4 than at sites 1-3 combined ($F_{3, 40}$ = 6.694, p = 0.001; Table 6.17). Overall, *G. pulex* abundance was threefold higher in July than in other months, but temporal change was not significant ($F_{2.395, 102.988}$ =

1.502, p = 0.225; Table 6.16). The interaction with site was, however, significant ($F_{8.065,\ 107.532}$ = 3.028, p = 0.004), and the July peak resulted in significant temporal change at site 4 ($F_{1.615,\ 17.761}$ = 4.155, p = 0.040).

6.7 Benthic invertebrate use of the hyporheic zone

To determine the extent to which the hyporheic zone habitat was used by benthic invertebrates, the hyporheic proportion of the total (benthic + hyporheic) community was calculated for each month (see Section 4.9.6; aim 2, objective 3). Four predominantly benthic non-insect macroinvertebrate taxa were selected for this analysis: Oligochaeta, Sphaeriidae, Hydracarina and *Gammarus pulex*. *G. pulex* abundance was low (a total of 67 individuals) but was included to allow comparison with the River Lathkill. Chironomidae larvae were also included due to their dominance in benthic and hyporheic communities, as were Simuliidae, due to preliminary observation of interesting patterns; these results should be treated with caution due to the complicating influence of seasonal adult emergence.

Total invertebrate abundance

The hyporheic proportion of TIA varied between sites, being comparable at sites 1, 2 and 3 (0.7-0.11 ± 0.01-0.02) and higher at site 4 (0.16 ± 0.02; $F_{3,\ 12}$ = 5.320, p = 0.015). Overall, the hyporheic proportion of TIA was lowest in September and peaked in June but differences between months were not significant ($F_{4,\ 44}$ = 0.446, p = 0.775), and nor was the interaction with site (Table 6.18).

Chironomidae larvae

The hyporheic proportion of the Chironomidae population was lowest at site 2 (0.1 ± 0.02) and was particularly high at site 4 (0.2 ± 0.03); spatial variation was not significant ($F_{2,\ 9}$ = 2.887, p = 0.108). Considering all sites, the hyporheic proportion varied little between months (Table 6.18). Greater variation was observed at site 4, the proportion increasing between May (0.13 ± 0.02) and July (0.23 ± 0.12) then remaining high in subsequent months; however, temporal variation was not significant at this site or overall.

Table 6.18: The hyporheic proportion of the total (benthic + hyporheic) invertebrate community present on the River Glen, May to September 2008.

	May	June	July	August	Sept	Temporal change
TIA	0.10 ± 0.02	0.14 ± 0.03	0.011 ± 0.02	0.12 ± 0.02	0.09 ± 0.03	ns
Chironomidae	0.16 ± 0.03	0.15 ± 0.03	0.12 ± 0.03	0.16 ± 0.03	0.13 ± 0.03	ns
Gammarus pulex	0.05 ± 0.03	0.17 ± 0.09	0.04 ± 0.02	0.09 ± 0.06	0.09 ± 0.06	ns
Hydracarina	0.02 ± 0.01	0.14 ± 0.04	0.11 ± 0.04	0.26 ± 0.08	0.19 ± 0.05	ns
Oligochaeta	0.07 ± 0.03	0.10 ± 0.03	0.13 ± 0.04	0.10 ± 0.02	0.13 ± 0.06	ns
Simuliidae*	0.25 ± 0.12	0.60 ± 0.09	0.35 ± 0.07	0.07 ± 0.04	0.01 ± 0.01	**
Sphaeriidae	0.01 ± 0.01	0.15 ± 0.09	0.16 ± 0.07	0.11 ± 0.07	0.07 ± 0.03	ns

*West Glen sites (1 and 2) only

Oligochaeta

The hyporheic proportion of the oligochaete population varied from a low of 0.05 ± 0.02 at site 2 up to 0.18 ± 0.05 at site 4, but spatial variability was not significant. Temporally, the proportion of oligochaetes within the hyporheic zone was lowest in May and highest in July and September, but temporal changes were not significant ($F_{2.709, 26.797} = 0.682$, $p = 0.556$; Table 6.18).

Sphaeriidae

Sphaeriids were virtually absent from the hyporheic zone of site 2, whilst the hyporheic proportion of this taxon peaked at 0.17 ± 0.07 at site 3; spatial variability was not significant ($F_{3, 12} = 2.008$, $p = 0.167$). Temporally, the hyporheic proportion of the sphaeriid community was particularly low in May and considerably higher in June and July, but differences between months were not significant overall ($F_{2.294, 25.239} = 1.154$, $p = 0.337$; Table 6.18), or at site 1 (where the taxon was particularly abundant; Tables 6.12 and 6.17).

Hydracarina

The proportion of the total Hydracarina population inhabiting the hyporheic sediments varied between sites ($F_{3, 12} = 5.494$, $p = 0.013$), being comparable at sites 2-4 (0.9-0.12 ± 0.04) and higher at site 1 (0.27 ± 0.06). Temporally, the proportion of the Hydracarina population resident within the hyporheic zone was particularly low in May and more than tenfold higher in August, but differences between months were not significant ($F_{4, 44} = 2.159$, $p = 0.089$; Table 6.18).

Gammarus pulex

The hyporheic proportion of the *G. pulex* population varied between sites, being particularly high at site 4 (0.15 ± 0.07; $F_{3, 12} = 3.639$, $p = 0.045$). Overall, the hyporheic proportion was twice as high in June as in any other month and was lowest in July, although patterns of temporal change were site specific; at site 4, for example, the proportion increased each month to a August-September peak. However, abundances were low throughout and no temporal changes were significant (Table 6.18).

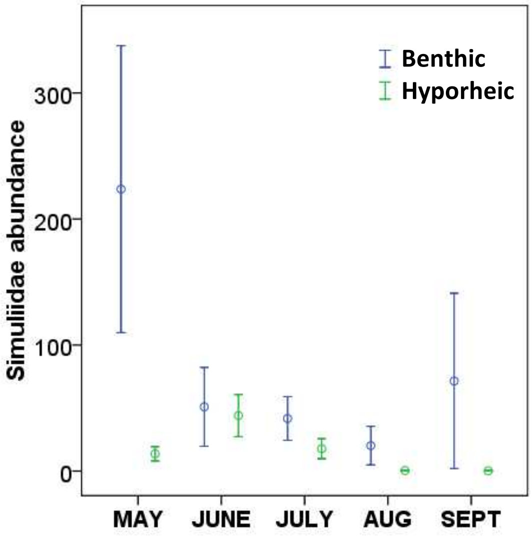


Figure 6.19: Mean ± 1 SE Simuliidae abundance in the benthic (0.1 m^{-2}) and hyporheic (6 L^{-1}) sediments, from which temporal change in hyporheic proportion can be inferred.

Simuliidae

Simuliidae larvae were largely restricted to the West Glen so only data from sites 1 and 2 were analysed. At these sites, the hyporheic proportion of the community was considerably higher in June than in any other month (the only taxon for which hyporheic exceeded benthic proportion in any month; Table 6.18), reflecting both decreased benthic abundance and increased hyporheic abundance (Figure 6.19); this was the only common taxa for which temporal change in the hyporheic proportion was significant ($F_{4, 28} = 9.618$, p <0.001; see also Figure 7.4).

6.8 Invertebrate-environment relationships

Canonical correspondence analysis (CCA) is used to examine relationships between invertebrate communities and environmental parameters, in both the benthic and the hyporheic zones (aim 2, objective 4). Pearson correlation coefficients were also calculated to assess relationships between environmental variables and abundance of common taxa in both benthic and hyporheic sediments, but this analysis yielded little useful information and is not presented.

6.8.1 Canonical correspondence analysis: benthic community

Sufficient data were available for the inclusion of three hydrological variables (depth, width, velocity) and five water chemistry variables (temperature, pH, conductivity, DO (concentration and % saturation)). Preliminary analyses were conducted using forward selection procedures with 999 iterations of the Monte Carlo random permutation test, to identify autocorrelated and non-significant variables. Performance of the model was enhanced by excluding DO (concentration) and retaining the remaining seven variables; all included variables then made a significant contribution to the explanatory power of the model ($p \leq 0.006$) and redundancy in the dataset was minimised (variance inflation factors ≤1.99). No arch effect was observed and detrending was therefore not necessary.

Spatial variability

Monte-Carlo tests indicated a significant contribution of both the first axis and the trace to model performance ($p = 0.001$) although the F-ratio was higher for axis 1 ($F = 10.311$) than for the trace ($F = 5.772$). Eigenvalues were low, suggesting that the environmental gradients influencing the community were weak (Table 6.19).

Axis 1 explained 12.5 % of the variance and was primarily correlated with depth and width, as well as pH (Table 6.19). Samples plotted according to site-specific differences in these variables, with little overlap between deep, low-pH perennial site 1 and shallow, high-pH intermittent sites 2 and 4 (Figure 6.20(i)). Differences in site axis scores were highly significant ($F_{3,76} = 58.481$, $p < 0.001$). Whilst many abundant taxa were ubiquitous and plotted at the centre of the species plot, those with individual site associations were positioned accordingly, for example *P. antipodarum* was most abundant at site 1 and absent from sites 2 and 4, and scored highly on axis 1 (Figure 6.20(ii)). The second axis explained 9.8 % of the species data variation, was also most strongly correlated with depth and width. This axis primarily reflected tributary-specific hydrological variability, with deeper, faster-flowing West Glen sites having lower scores (Figure 6.20(i)). Other relevant variables included temperature, with particularly high temperatures at site 4 reflected by positioning of these samples exclusively in the positive quadrants of axis 2. The majority of common taxa plotted near the centre of axis 2, although *S. ignita* was largely restricted to site 2 and had a low axis score (Figure 6.20(ii)).

Temporal variability

CCA was repeated with site as a covariable to examine general patterns of temporal change (Figure 6.21; Table 6.19). Monte Carlo tests indicated that explanatory power of variables associated with both the first axis and the trace was significant (p = 0.001), although F –ratios were low for both axis 1 (F = 5.675) and the trace (F = 3.114). All eigenvalues were low, indicating that environmental gradients related to combinations of these variables were weaker than those related to site-specific variability (Table 6.19).

Axis 1 explained 6.9 % of the variance in the species data and had highly significant correlations with velocity and temperature. Axis 1 scores decreased between May and June and fell again in July then remained similar and low for the remainder of the study period ($F_{4, 75}$ = 29.243, p <0.001; Figure 6.21(i)); these changes reflected high temperatures in July and August and lower flow velocities from July onwards. All common taxa (>1 % TIA) plotted towards the centre of axis 1, indicating their occurrence throughout the range of environmental conditions, whilst taxa at the axis extremities represented very few (≤2) individuals (Figure 6.21(ii)). Axis 2 explained a further 4.9 % of the species data variance, and was significantly correlated with temperature and conductivity. Axis 2 scores were moderate in May and June, peaked in July then declined to a five-month low in September ($F_{4, 75}$ = 7.562, p <0.001). Again, changes were partly due to peak temperatures in July and August,

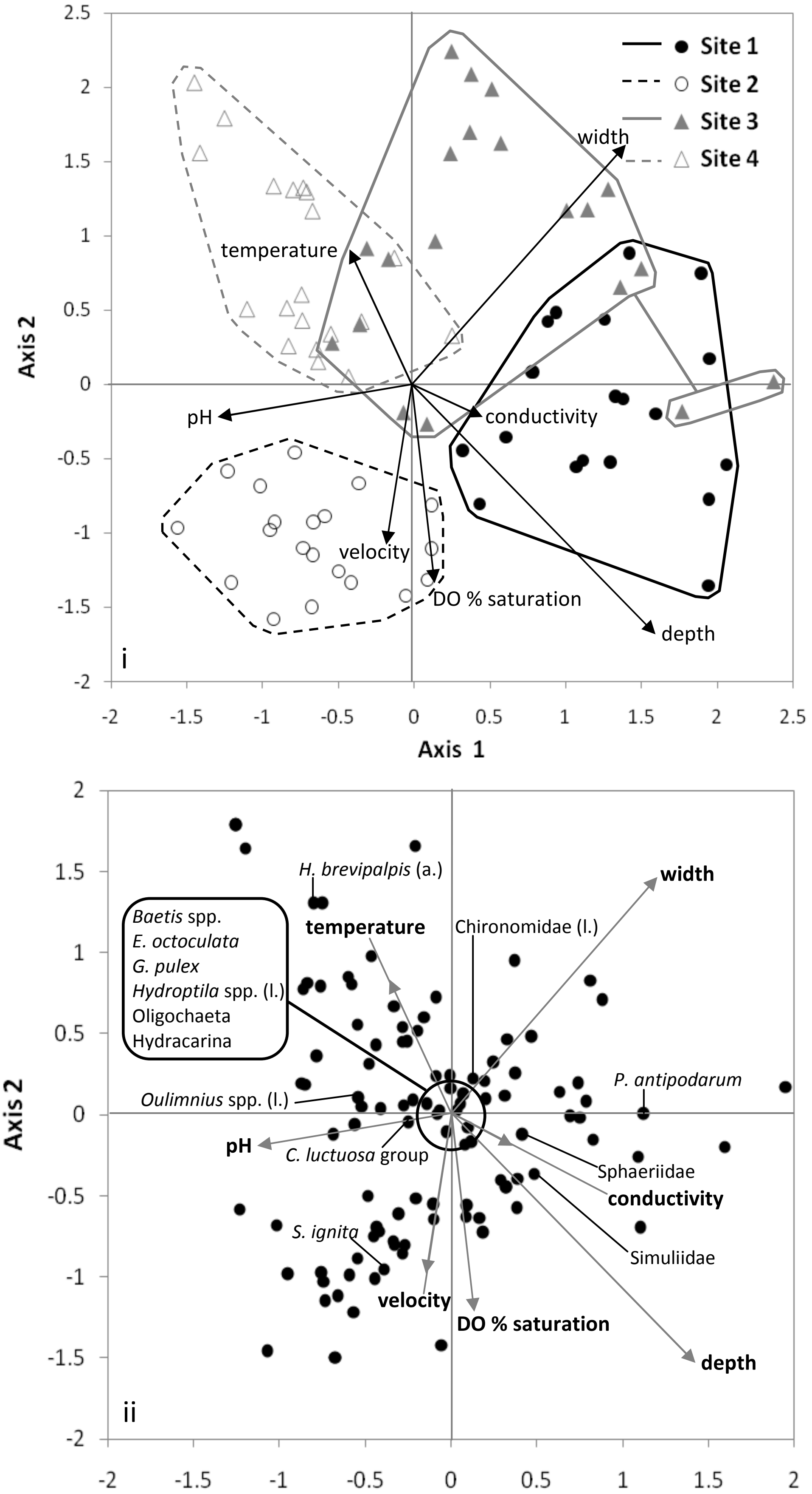


Figure 6.20: Canonical correspondence analysis ordination of benthic invertebrate and surface water data. Spatial variability: i) sample-environment biplot. Key: circles = West Glen; triangles = East Glen; ii) species-environment biplot.

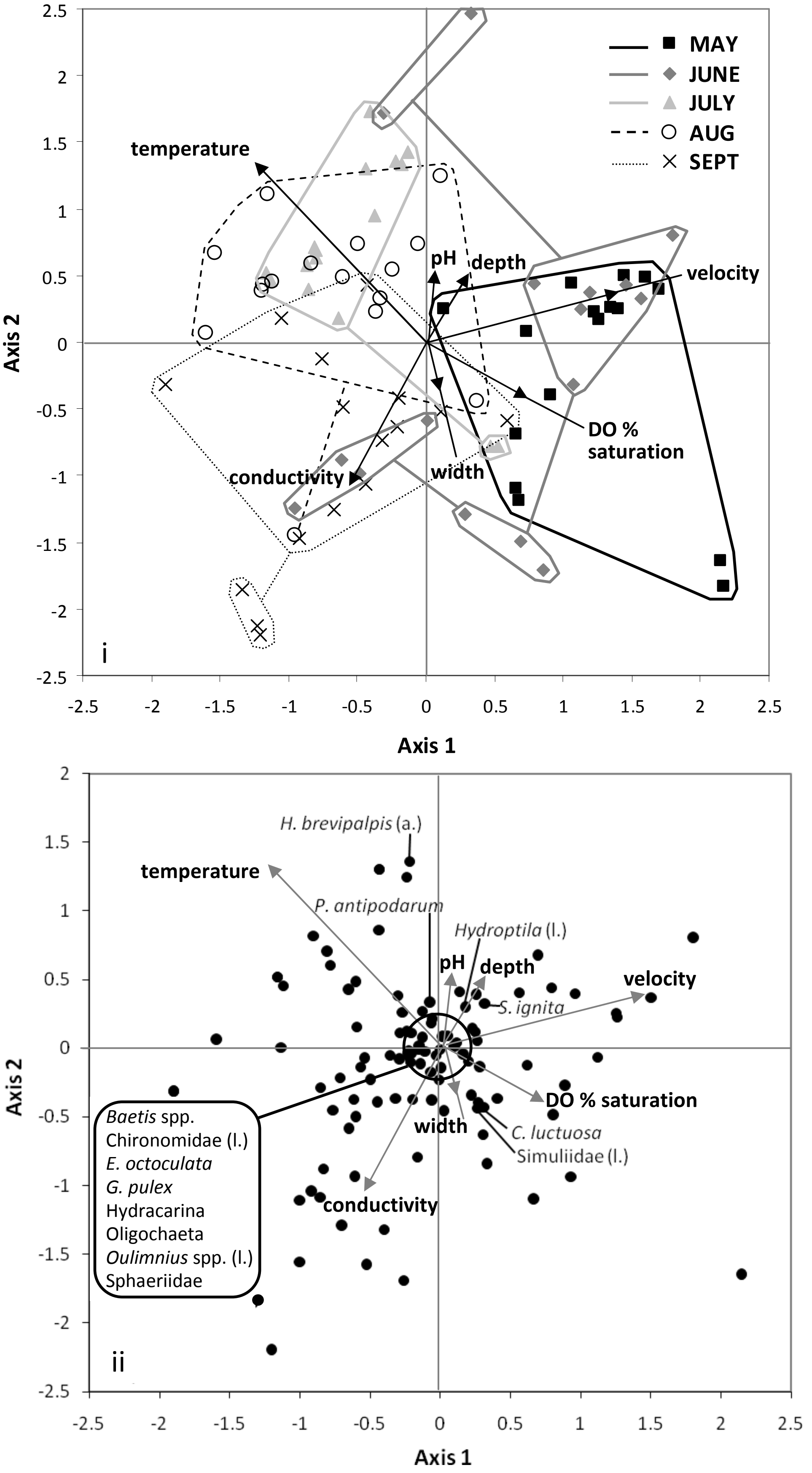


Figure 6.21: Canonical correspondence analysis ordination of benthic invertebrate and surface water data, with site as a covariable. Temporal variability: i) sample-environment biplot; ii) species-environment biplot.

and also reflected high conductivity in September. All common taxa plotted near the centre of axis 2, whilst semi-aquatic taxa (e.g. *Helophorus brevipalpis*) had high axis scores, reflecting their occurrence after habitat contraction in July (Figure 6.21(ii)).

Table 6.19: Summary of canonical correspondence analysis of benthic invertebrate community and environmental data from the River Glen

	Eigenvalues				**Cumulative % variance of species data**				**Cumulative % variance of species-environ. relation**			
Axis	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
ALL SITES	0.167	0.131	0.057	0.048	12.5	22.3	26.6	30.2	34.8	62.2	74.0	84.0
ALL SITES (covariable)	0.067	0.047	0.037	0.037	6.9	11.8	15.7	19.5	29.6	50.6	67.2	83.5

The original CCA (Figure 6.20) highlighted the primary importance of site-specific variability in determining community composition, with water depth and wetted width proving most influential; this analysis found no significant difference in axes scores between months ($p \geq 0.09$). Repetition of the analysis with site as a covariable (Figure 6.21) highlighted the importance of flow velocity and temperature in determining community composition, with both depth and width remaining relatively constant over time. There was, however, considerable overlap between months and spatial variability remained particularly important in June, when samples formed largely site-specific clusters that spanned the length of axis 2 (Figure 6.21(i)).

6.8.2 Canonical correspondence analysis: hyporheic community

Data was available for five environmental variables: temperature, pH, conductivity, DO concentration and DO % saturation. Preliminary analysis showed the DO measures to be autocorrelated (variance inflation factors ≥27.9) and concentration was therefore excluded. Monte Carlo tests indicated that pH did not contribute to the explanatory power of the model ($F = 1.39$, $p = 0.1180$) and this variable was therefore also excluded. No arch effect was observed and detrending was therefore not required.

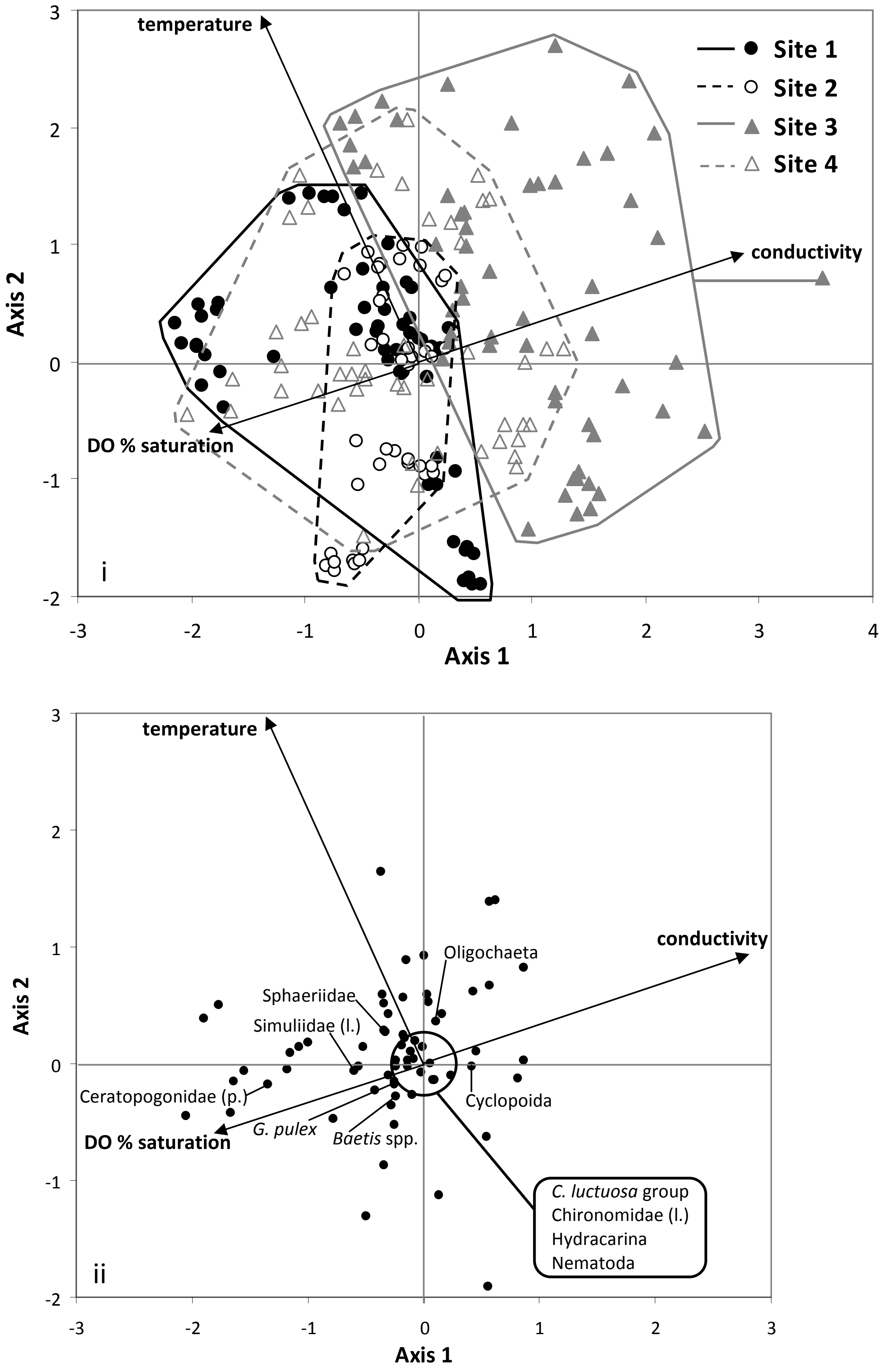


Figure 6.22: Canonical correspondence analysis ordination of hyporheic invertebrate and water chemistry data. Spatial variability: i) sample-environment biplot. Key: circles = West Glen; triangles = East Glen; ii) species-environment biplot.

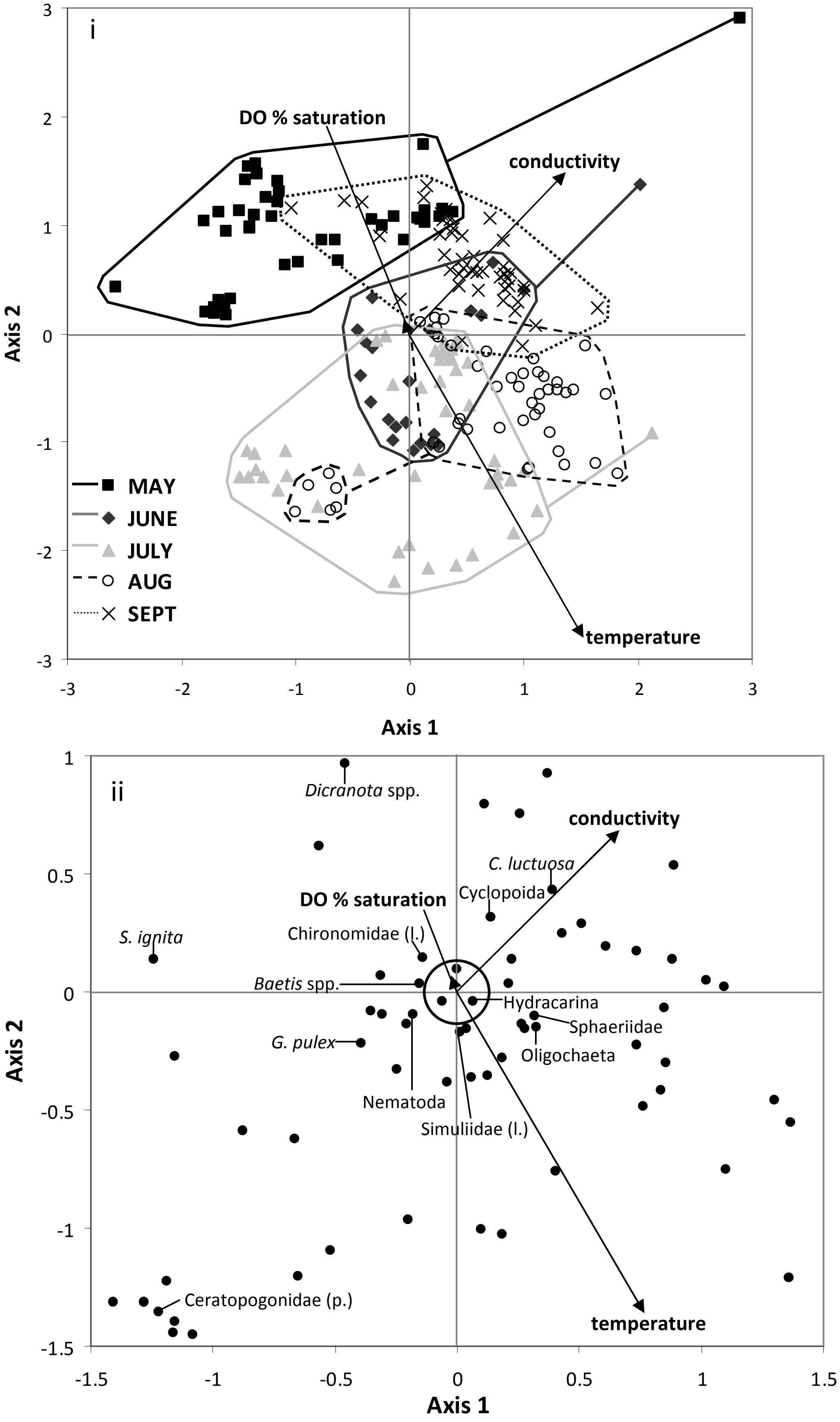


Figure 6.23: Canonical correspondence analysis ordination of hyporheic invertebrate and water chemistry data, with site as a covariable. Temporal variability: i) sample-environment biplot; ii) species-environment biplot.

Spatial variability

Monte Carlo tests found both the first canonical axis and the trace to explain a significant amount of the variation in the dataset (*F*-ratio = 5.184, *p* = 0.002 and *F*-ratio = 3.598, *p* = 0.002 respectively). The contribution of all three variables to the explanatory power was similar (*F*-ratios = 3.07-4.05, *p* = 0.001). However, eigenvalues were very low, indicating weak environmental gradients (Table 6.20).

Axis 1 explained only 2.5 % of the variation in species data and was significantly correlated with all variables. Axis 1 scores differed between sites ($F_{3, 202} = 39.914$, $p < 0.001$); *post-hoc* multiple-comparison tests indicated that this was solely due to higher scores at site 3 (Figure 6.22(i)). Considerable overlap was observed between all other sites, with site 2 samples forming a distinct cluster near the axis origin and site 4 samples being widely distributed. Many common taxa were ubiquitous and plotted at the centre of the ordination, whilst Ceratopogonidae pupae, which occurred only at site 4, and Simuliidae larvae, which were not observed at site 3, plotted in the negative quadrants (Figure 6.22(ii)). The high scoring axis 1 outlier (Figure 6.22(i)) had unusually high conductivity whilst the community featured the highest densities of both Chironomidae larvae and Cyclopoida copepods recorded, explaining the positive score of the latter taxon.

Axis 2 explained only 1.3 % of the species data variation but had a particularly strong correlation with temperature. Despite considerable overlap, axis 2 scores did differ between sites ($F_{3, 202} = 7.814$, $p < 0.001$; Figure 6.22(i)), and as for axis 1, this spatial variability reflected a distinction between site 3 and all other sites. Mean temperature was moderate at site 3, but was particularly variable and samples with the highest axis 2 scores were characterised by higher temperatures, low DO availability, and particularly depauperate communities. Most taxa plotted towards the centre of axis 2, whilst those taxa with the most extreme scores were represented by single individuals (Figure 6.22(ii)).

Temporal variability

Within-site clustering observed on the CCA ordination examining spatial variability suggested the importance of temporal change, and this was examined further by including site as a covariable (Figure 6.23). All three variables made a significant contribution to the explanatory power of the model, although significance was marginal for DO % saturation (*F*-ratio = 1.54, *p* = 0.05). Monte-Carlo tests also indicated the significance of both axis 1 (*F*-ratio = 3.740, *p* = 0.008) and the trace (*F*-ratio = 2.768, *p* = 0.002), although low eigenvalues indicated weak environmental gradients (Table 6.20).

Axis 1 explained only 1.8 % and axis 2 a further 1.5 % of the community variation (Table 6.20); both axes were most strongly correlated with temperate and also had significant correlations with conductivity. Temporal change was significant on both axis 1 ($F_{4, 201}$ = 30.743, *p* <0.001) and axis 2 ($F_{4, 201}$ = 109.329, *p* <0.001). Axis 1 scores were low in May, moderate in June and July and high in August and September (Figure 6.23(i)); this pattern could not be related to any single environmental variable, and appeared to be primarily influenced by seasonal declines in insect abundance, hence the positioning of Chironomidae, *Baetis* spp. and *S. ignita* in the top left quadrant (Figure 6.23(ii)). Axis 2 scores decreased between May and July then rose in each subsequent month, closely reflecting changes in hyporheic water temperature. An outlying group of August samples reflected unusually low conductivity at site 3, whilst the invertebrate communities of these samples were diverse (Figure 6.23).

Spatiotemporal variability

Comparison of the two ordinations (Figures 6.22 and 6.23) indicated that temporal changes in community composition were more pronounced than spatial differences; this contrasts with the distinct site-specific assemblages observed for the benthic community. Temporal change was particularly apparent at individual sites 1 and 2; notably, reference to the raw data revealed the site 1 low-scoring axis 1 cluster (Figure 6.22(i)) to reflect high hyporheic abundance of several predominantly benthic taxa (particularly Simuliidae larvae) in June. The significant correlations between axis scores and temperature, as well as the positioning of insect taxa away from the origin, suggested that temporal changes were largely seasonal rather than related to hydrological variability.

Table 6.20: Summary of canonical correspondence analysis of hyporheic invertebrate community and environmental data from the River Glen

	Eigenvalues				Cumulative % variance of species data				Cumulative % variance of species-environ. relation			
Axis	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
ALL SITES	0.099	0.052	0.049	0.464	2.5	3.8	5.1	16.8	49.3	75.4	100	0
ALL SITES (covariable)	0.066	0.051	0.026	0.432	1.8	3.3	4.0	16.1	46.0	81.8	100	0

6.9 Spatial variability in invertebrate habitat

To help explain use of the hyporheic zone by benthic invertebrates, two 'stable' habitat parameters that can affect hyporheic invertebrate community composition are investigated: the sediment grain size distribution and the direction and strength of hydrologic exchange (aim 2, objective 5). Whilst both of these variables are, in fact, temporally variable (in particular during spate events), they are relatively constant compared to the hydrological and water quality parameters measured each month.

6.9.1 Sediment composition

To facilitate comparison with previous studies, the grain size distribution of McNeil samples collected from each site (see section 4.6.2) was expressed using a range of sediment size classes, from <63 µm to >8 mm (Table 6.21). Two sediment size classes differed between sites, <63 µm and 125-150 µm, both of which were similar at sites 2-4 but higher at site 1 ($F \geq 5.756$, $p \leq 0.021$). On-site observations at site 1 indicated that sample points 1 and 4 were situated in a clay-rich area, whilst point 2 was located in a more heterogeneous area where clay was only present at a depth of ~20 cm (see Appendix 6 for sampling point locations). A clay layer was also present near the base of all site 3 samples; the percentages for the finest size classes presented in Table 6.21 being underestimates due to aggregation of finer sediments during oven drying. Site 2 was characterised by the highest percentage of larger (>2 mm) grains.

Table 6.21: Grain size distribution of sediment samples from River Glen sites 1-4

	% sediment in each size class											
	Site 1 sample areas			Site 2 sample areas			Site 3 sample areas			Site 4 sample areas		
Sediment size class	**1**	**2**	**4**	**1**	**2**	**4**	**1**	**2**	**4**	**1**	**2**	**4**
<63 µm	4.9	1.3	4.6	0.7	0.6	0.4	0.6	1.0	0.6	0.4	0.4	0.4
63-125 µm	6.9	2.3	1.9	1.7	2.2	1.3	1.4	2.0	1.7	0.7	0.8	0.9
125-250 µm	9.5	3.8	9.5	2.5	3.4	1.6	2.7	3.4	5.0	2.3	2.1	2.0
250-500 µm	**14.6**	**12.1**	**13.2**	5.1	6.6	3.9	7.1	**25.1**	**12.9**	**12.9**	**10.9**	**11.8**
0.5-1 mm	**18.1**	**13.4**	**12.7**	7.7	**12.2**	7.9	8.7	**17.9**	9.3	7.2	**13.0**	**15.3**
1-2 mm	**14.9**	9.9	**17.2**	**13.5**	**11.8**	**11.0**	7.7	5.5	7.8	5.8	**12.6**	**10.0**
2-4 mm	9.7	8.7	**17.4**	**15.4**	**12.6**	**14.2**	**11.1**	6.6	**10.6**	9.3	**12.8**	**10.3**
4-8 mm	4.6	**14.2**	8.7	**14.4**	**15.8**	**15.9**	**20.4**	**12.9**	**16.8**	**16.1**	**16.3**	**12.7**
>8 mm	**16.8**	**35.3**	8.7	**39.0**	**34.9**	**43.8**	**40.3**	**25.7**	**35.4**	**45.2**	**31.1**	**36.6**

Sediments not characterised at sampling area 3 of any site. Size classes comprising >10 % of the sediment weight in bold; dominant size class is highlighted.

Pearson correlation coefficients were calculated to assess the effects of relatively constant sediment composition on hyporheic community metrics (Table 6.22). Invertebrate abundance had weak negative correlations with the three finest sediment size classes, this being significant for the 125-250 µm fraction (Table 6.22). Taxon richness was also negatively correlated with these fine sediment classes, this also being most pronounced for the 125-250 µm fraction. Equally, there was a highly significant positive correlation between the number of taxa in the hyporheic zone and the percentage of grains >8 mm. Community dominance appeared unaffected by the grain size distribution, although additional analyses comparing dominance with *cumulative* percentages of sediment below successive thresholds indicated a weak, non-significant but consistent pattern of higher dominance values in sediments with a greater proportion of fine (particularly <1 mm) grains (Table 6.22). Analysis of data from individual depths did not reveal any other significant patterns.

Table 6.22: Pearson correlation coefficients between sediment composition and hyporheic invertebrate community metrics

Sediment size class	**Abundance[1]**	**Taxon richness[1]**	**Dominance[2]**	**Cumulative sediment size class**	**Dominance[2]**
<63 µm	-0.206	-0.369**	0.037	**<63 µm**	0.035
63-125 µm	-0.201	-0.204	-0.056	**<125 µm**	0.031
125-250 µm	-0.256*	-0.422**	0.102	**<250 µm**	0.060
250-500 µm	0.090	0.000	0.236	**<500 µm**	0.175
0.5-1 mm	0.176	-0.037	0.150	**<1 mm**	0.179
1-2 mm	-0.019	-0.246	-0.126	**<2 mm**	0.129
2-4 mm	-0.198	-0.222	-0.166	**<4 mm**	0.093
4-8 mm	0.046	0.179	0.025	**<8 mm**	0.132
>8 mm	0.122	0.345**	0.132	**-**	-

[1]Combined from all hyporheic depths; [2]Berger-Parker dominance index, mean of all hyporheic depths; *p ≤0.05; ** p ≤0.01

6.9.2 Hydrologic exchange

Monthly mini-piezometer data were collected from July onwards at sites 2-4, but measurements were only taken in September at site 1. The installed equipment was simplistic, and was intended to allow determination the direction of exchange and rough estimation of its strength; it is not, however, appropriate to attempt calculation of the precise vertical hydraulic gradient.

Site 4 measurements indicated strongly downwelling water in all months; such readings could reflect the presence of sediments with low hydraulic conductivity between the piezometer base and the sediment surface (Boulton, 2007b). However, site 4 commonly dries during the summer months, in part due to transmission losses through the streambed (Rushton and Tomlinson, 1999), and these readings are therefore considered accurate. Site 2 has also experienced recent streambed drying due to karstic sinkholes and was predominantly downwelling, although the strength of this exchange generally weakened during the study, with some upwelling water being recorded in September.

Hydrologic exchange was also weak- to moderately-downwelling at perennial site 1 in September, although functioning of the mini-piezometers may have been compromised by clay layers (see section 6.9.1). Similarly, stable to moderately-upwelling water was recorded at perennial site 3 (in what is considered a gaining reach; Rushton and Tomlinson, 1999), but the strength of exchange may have been underestimated due to the presence of clay in some parts of the substrate.

6.10 Discussion

In this section, the results of the sampling campaign on the River Glen are described in relation to the thesis aims (section 1.2). Environmental conditions are considered as potential environmental stressors, then the benthic invertebrate community response to each identified stressor is considered, with particular focus on the use of the hyporheic refugium. Both habitat-related and disturbance-related factors that may have limited refugium use are emphasized.

6.10.1 Identification of potential environmental stressors

The first aim of this chapter (section 6.2) was to identify changes in surface hydrology, hydrologically-mediated environmental conditions and related biotic factors with the potential to stress benthic invertebrates.

Variation in surface hydrology in long-term context

The hydrological conditions on the Glen included a series of high-flow events between the May and June sampling dates (Figure 6.2). These were potentially adverse hydrological conditions for benthic invertebrates. However, flow duration analysis (Figures 6.3 and 6.4) demonstrated that the highest discharges recorded during the study were unexceptional and higher-magnitude spates occurred during the preceding winter (data not shown); the invertebrate community should therefore be adapted to resist such events (Lytle and Poff, 2004). Flow duration analysis also indicated that the lowest flows were greater than is typical, although the hydrograph (Figure 6.2) showed that site 4 experienced short-duration streambed drying in both late July and early September. Hydrological conditions therefore remained moderate compared with long-term average conditions, but localised streambed drying may have been a direct invertebrate stressor at site 4 in later months.

Effect of flow variability on instream habitats

Discharge data alone is insufficient to characterise effects of flow variability on benthic invertebrate habitat, and localised development of adverse conditions was observed. Firstly, flow velocities were low at site 3 (Figure 6.5(ii)), particularly from June when localised ponding occurred; these low velocities reduced habitat heterogeneity and were associated with low oxygen and high fine sediment concentrations, both of which limit habitat suitability and reduce survival for many benthic taxa (Wood and Armitage, 1997; Connolly *et al*., 2004). Secondly, width and depth were particularly low at site 4 in July (Figure 6.5(i) and (iii)). A low water volume can increase the influence of external factors (e.g. insolation and groundwater; Dewson *et al*., 2007a), but minor increases in temperature and associated reductions in dissolved oxygen availability in July were unlikely to have had biotic effects (Murdoch *et al*., 2000). Of greater potential importance was the reduction in habitat availability (Table 6.5), since habitat contraction can concentrate benthic invertebrates into a smaller submerged area (Fritz and Dodds, 2004; Dewson *et al*., 2007b). This habitat reduction was followed by complete loss of surface water, which represents a critical threshold (*sensu* Boulton, 2003) after which impacts on aquatic fauna are severe (e.g. Smock *et al*., 1994; Fritz and Dodds, 2004); this temporary loss of surface water is likely to explain particularly low hyporheic oxygen availability in August (September data not available).

Potential effects of flow variability on biotic interactions

Whilst no common non-insect taxon experienced significant temporal changes in overall abundance following habitat contraction at site 4, a notable fourfold increase in *Gammarus pulex* abundance was observed between June and July. *G. pulex* is a highly competitive taxon which can potentially influence community composition through both competition and predation (Dick *et al*., 1995), however, population densities remained much lower than reported by some studies (Mortensen, 1982; Crane, 1994), and as such the taxon is unlikely to have caused a significant intensification of biotic interactions (e.g. predation). Similarly, the Hydracarina occurred at particularly high densities in July at site 4, however Hydracarina primarily consume meiofauna and so are unlikely to have impacted upon macroinvertebrate populations (Cassano *et al*., 2002). Whilst many other taxa also occurred at high abundance at site 4 in July, total invertebrate densities did not approach values recorded in May, when habitat availability was high. Habitat contraction and the concurrent increase in population densities may therefore have caused only a moderate increase in the strength of biotic interactions.

6.10.2 Benthic community response to hydrological variability

The second aim of this chapter (section 6.2) was to examine benthic community responses to factors identified as potential stressors, and community composition in the benthic sediments is considered in this section.

Temporal change in benthic community composition

Multivariate ordinations indicated that environmental and benthic community differences were generally more pronounced between sites than between months, suggesting that temporal change in hydrological conditions had little impact on community composition. Considering the series of small spates, such events are recognised as disturbance events which can have pronounced impacts on benthic invertebrate communities (Death, 2008). However, spate magnitude and duration are determinants of detrimental impacts and some previous studies have shown low-intensity spates to have little impact on benthic communities (Robinson *et al*., 2004). In the River Glen, only the Chironomidae were observed to decline significantly in abundance between May and June, and this is may reflect seasonal adult emergence (Learner and Potter, 1974); similarly, the Simuliidae experienced a considerable decline in abundance, which could either reflect displacement by high flows or adult emergence (Table 6.11).

At the other hydrological extreme, reduced flow at site 4 in July coincided with increases in the abundance of most common taxa, indicating concentration of stable populations into a contracting habitat area. In addition, taxon richness at site 4 peaked in July following exposure of marginal benthic sediments, due to the arrival of Coleoptera associated with exposed sediments (i.e. *Agabus didymus, Helophorus brevipalpis*; Stubbington *et al*., 2009b). Despite habitat availability returning to ‘normal’ at site 4 in August, severe reductions were observed in both taxon richness and TIA, the latter reflecting considerable reductions in a diverse range of taxa including the Hydracarina (>99 % decline), *Baetis* spp. (>99 %), *G. pulex* (94 %), Chironomidae (89 %) and *Asellus aquaticus* (88 %). The severity of these reductions almost certainly relate to short-duration complete streambed drying in late July. The reduced abundances generally declined further in September, which is assumed to relate to the second drying event between August and September sampling. Interestingly, reductions in both richness and abundance increased community diversity due to a disproportionate impact on dominant taxa (Death, 2008; Mesa, 2010). Only two common taxa appeared unaffected by the loss of surface water: the Oligochaeta, a group including many species with physiological adaptations to drying

(Kenk, 1949; Williams, 2006), and *Bithynia leachii*, a prosobranch gastropod which can seal its operculum to prevent water loss.

6.10.3 Benthic invertebrate use of the hyporheic zone

In this section, evidence for the hyporheic refuge hypothesis (Williams and Hynes, 1974) is examined for each condition previously identified as a potential stressor.

Use of the hyporheic zone following the spate

Sampling in the current investigation was conducted at monthly intervals, with discharge data indicating that samples were collected several days after the spate disturbances (Figure 6.2). Even field studies specifically targeting spates are restricted to sampling in the days following an event (e.g. Dole-Olivier *et al*., 1997; Olsen and Townsend, 2003), thus limiting the extent to which conclusions can be drawn regarding refugium use *during* high flows. In the Glen, whilst the spates affected all sites, these events did not have detectable detrimental impacts on the benthic community, with the possible exceptions of the Chironomidae and Simuliidae. The need for migration into the hyporheic refugium was therefore limited to few taxa. Of these taxa, a moderate decline in the benthic abundance of the Simuliidae was accompanied by a significant increase in its hyporheic abundance and in the hyporheic proportion of the total population (Figure 6.19; Table 6.18); this provides tentative evidence of Simuliidae either actively migrating or being passively washed into the hyporheic zone during high flows.

Use of the hyporheic zone during habitat contraction

At site 4, two potential invertebrate stressors were identified: habitat contraction and an associated increase in benthic population densities (and therefore biotic interactions) in July; and recent streambed drying in August and September. In July, an increase in benthic abundance during habitat contraction was accompanied by some rise in hyporheic population densities for many taxa, including Chironomidae, Oligochaeta, *Baetis* spp., Hydracarina and *G. pulex*. However, in no case was this associated with an increase in the hyporheic proportion of the total population. This is unsurprising, since conditions appeared to remain favourable in the contracting habitat, and hyporheic increases are considered passive range extension rather than active refugium use (Wood *et al*., 2010; see section 7.11 and Figure 7.4). Nonetheless, the moderate increases in hyporheic abundance suggested that the hyporheic zone at site 4 was suitable for inhabitation by several benthic taxa.

Use of the hyporheic zone after streambed drying

The hyporheic zone is proposed to act as a refugium during streambed drying due to the retention of free water (Boulton *et al*., 1992). However, previous evidence of this refugial role is equivocal, with some studies noting active migrations to greater depths following drying (Boulton *et al*., 1992; Cooling and Boulton, 1993; Griffith and Perry, 1993), whilst others have observed no increase in invertebrate abundance (Boulton, 1989; Boulton and Stanley, 1995; Del Rosario and Resh, 2000). In the Glen, severe reductions in benthic abundance in August and September were accompanied by declines in the hyporheic abundance of common taxa including Chironomidae, Hydracarina, *G. pulex* and *Baetis* spp. In contrast, the hyporheic abundance of the Oligochaeta remained stable in July and August then increased in September, whilst benthic abundances fell; the associated increase in the hyporheic proportion of the oligochaete population was not, however, significant. Therefore, no conclusive evidence supporting the hyporheic refuge hypothesis was recorded on the Glen, although oligochaetes may have migrated into the hyporheic zone during drying events to enhance survival. This taxon is morphologically suited to interstitial environments and species are commonly tolerant of low oxygen availability (e.g. Extence *et al*., 1987); its capacity to exploit the hyporheic zone refugium is therefore high. Regardless of whether hyporheic abundance and/or hyporheic proportion increased, any taxon that persisted within the hyporheic zone following streambed drying used these sediments to enhance survival, even if refugium use was passive (see section 7.11).

6.10.4 Suitability of the hyporheic sediments as a refugium.

The final objective set for this chapter was to relate spatial variability in hyporheic refugium use to the suitability of the hyporheic sediments as a benthic invertebrate habitat (section 6.2). Since little evidence of refugium use was recorded, conditions that may have lowered the hyporheic zone's refugial capacity are emphasized in the following discussion, with reference to historic flow characteristics, stable habitat variables (sediment grain size distribution, hydrologic exchange) and temporally variable water quality parameters.

Flow regime

Sites with historic perennial and intermittent flow had been selected to allow comparison of refugium use at sites with contrasting historic flow permanence regimes, this being considered a possible influence on hyporheic community composition and refugium use (Hose *et al*., 2005; Wood *et al*., 2010). However, relationships between flow permanence and environmental/community parameters were scarce and significant differences were instead tributary- or site-specific. It is therefore more appropriate to disregard the flow permanence distinction and consider site-specific flow regime characteristics and their potential impacts on refugial capacity.

Site 1 is perennial and retained flowing water; hydrologic exchange with the hyporheic zone should therefore have delivered oxygen and organic matter to the hyporheic sediments, promoting refugial capacity (Jones *et al*., 1995a; Brunke and Gosner, 1997). Site 2 was considered as intermittent but has dried recently only due to sporadic development of karst sinkholes (C. Extence, pers. comm.); this reach had previously been considered perennial (Maddock *et al*., 1995) and during the study had similar flow characteristics to adjacent site 1. Site 3 is also perennial, but may become ponded for long periods (Maddock *et al*., 1995), as occurred between June and September. As current velocities decline, fine sediment can be deposited and clog interstitial spaces, reducing hydrologic exchange and detrimentally affecting hyporheic water quality (Brunke and Gosner, 1997; Boulton, 2007a); therefore, the suitability of the hyporheic zone as a refugium at site 3 may have been low. Site 4 is truly intermittent and typically dries during the summer months. Much previous research into the hyporheic zone refugium has focussed on intermittent sites (e.g. Clifford, 1966; Boulton *et al*., 1992; Clinton *et al*., 1996; Fenoglio *et al*., 2006), reflecting the particular challenges faced by benthic invertebrates in such environments rather than suggesting that the hyporheic zone functions as a particularly effective refugium. In fact, the ability of the hyporheic zone to support benthic invertebrates following drying relies on the retention of free water, and refugial capacity is severely reduced if water is lost (Gagneur and Chaoui-Boudghane, 1991; Boulton and Stanley, 1995). No measurements of hyporheic water content were taken during this study; however, complete water loss was observed to a depth of >35 cm in 2009, indicating that transmission losses through the streambed can also cause hyporheic drying.

Sediment characteristics

Previous studies have noted negative relationships between the percentage of fine sediment in the substrate and community metrics (Richards and Bacon, 1994; Olsen and Townsend, 2003; Weigelhofer and Waringer, 2003), since fine sediments limit movement of invertebrates through interstices and also weaken hydrologic exchange, thus reducing water quality (Brunke, 1999; Sarriquet *et al.*, 2007). The proportion of fine sediment in bulk samples from the Glen were high at all sites compared with the fourth-order gravel-bed stream studied by Olsen and Townsend (2003) but comparable with the third-order sandstone stream of Weigelhofer and Waringer (2003) and accordingly, significant negative correlations between fine sediment classes and taxon richness were observed in the Glen (Table 6.22). The proportion of fine sediments was particularly high at site 1 (Table 6.21), with field observations indicating that clay layers affected two of four sampling areas at this site and were also present in parts of site 3, and suitability of the hyporheic zone as a refugium is likely to have particularly low in these areas.

Hydrologic exchange

The direction and strength of vertical hydrologic exchange (i.e. upwelling or downwelling water) is a major influence on the hyporheos (Boulton and Stanley, 1995; Davy-Bowker *et al.*, 2006). It has also been identified as influencing migrations of benthic invertebrates during disturbance events, with upwelling water impeding movement into the hyporheic zone whilst downwelling water facilitates migrations (Dole-Olivier *et al.*, 1997). In the Glen, strongly downwelling water should have promoted migrations into the hyporheic sediments at site 4, whilst upwelling water may have limited benthic invertebrate use of deeper sediments at site 3.

Water quality

Water quality was lower in the hyporheic zone compared with the surface channel at all sites, with significantly lower dissolved oxygen, higher temperatures and slightly higher conductivity being recorded and localised anoxia occurring at sites 3 and 4. Such observations are typical, with hyporheic oxygen availability being particularly low in upwelling zones (such as at site 3; Jones *et al*., 1995a) and following streambed drying (as at site 4; e.g. Smock *et al*., 1994). The hyporheic zone has therefore rarely been reported to function as a refugium from poor water quality (but see Jeffrey *et al*., 1986). In addition, several previous studies have noted negative correlations between community metrics and hyporheic dissolved oxygen concentrations (Boulton *et al*., 1997; Franken *et al.*, 2001).

6.10.5 Disturbance-related determinants of refugium use

In addition to environmental variability, refugium use can also be influenced by disturbance related parameters. Both Lancaster (2000) and Boulton *et al*. (2004), for example, attributed the failure of the hyporheic zone to act as a high-flow refuge to spate magnitude being too low to elicit a behavioural response; this reason is also given here to explain the lack of response to the Glen spates. Similarly, previous research considering refugium use following flow reduction has suggested that no migrations occurred due to conditions remaining favourable in the benthic sediments (James *et al*., 2008); again, such an explanation may be given for benthic invertebrates largely remaining in the surface sediments following habitat contraction at site 4. At the other extreme, disturbance magnitude may increase too quickly to allow a behavioural response, as has been observed in relation to high-magnitude spates (Imbert and Perry, 1999; Gayraud *et al*., 2000). Whilst a rapid rate of change has not previously been cited as determining refugium use following streambed drying, the flashy flow regime of the East Glen (and consequent rapid shifts between wet and dry conditions) may have limited refugium use.

6.11 Summary

Spate events, habitat contraction during low flows and streambed drying were all identified as potential stressors of the benthic community. However, closer inspection of benthic community composition indicated that both spates and habitat contraction had only localised and taxon-specific effects. Accordingly, little use of the hyporheic refugium was expected and evidence of active migrations was restricted to the Simuliidae during the spate. Streambed drying was identified as the major potential trigger of hyporheic refugium use. However, evidence of active refugium use during drying was limited to the Oligochaeta, whilst the hyporheic zone may have functioned as a passive refugium for a diverse range of taxa. A range of environmental and disturbance-related parameters have been discussed individually regarding their potential to limit the refugial capacity of the hyporheic zone. Whilst the failure of the hyporheic zone to act as refugium can sometimes be attributed to the overriding influence of an individual variable (Boulton, 1989 – high temperature; Smock *et al*., 1994 – anoxia; Olsen and Townsend, 2003 – high proportion of fine sediments), in many cases the combined influence of environmental variables outlined above determines refugium use (or lack thereof). Interplay between factors will be further discussed in Chapter 7.

7. Linking benthic invertebrate use of the hyporheic refuge to spatiotemporal variability in environmental conditions

7.1 Introduction

The previous two chapters have described results of sampling conducted on the River Lathkill (Chapter 5) and the River Glen (Chapter 6) to address thesis aims 1 and 2 (section 1.2). This chapter compares and contrasts these results in the context of existing literature, and this synthesis facilitates development of a conceptual understanding of the hyporheic zone as a refugium. First, observed conditions spanning the hydrological continuum (spates, low flows and streambed drying) are considered as stressors of the benthic community, through their effects on surface hydrology (aim 1, objectives 1 and 2), habitat availability (aim 1, objective 3) and water quality (aim 1, objective 4). Second, the effects of potential environmental stressors on the benthic fauna are examined, with particular attention paid to relationships between habitat availability and biotic interactions (aim 1, objective 5), and to the effects of identified stressors on benthic community composition (aim 2, objectives 1 and 2). The hyporheic zone is then considered as a refugium for benthic invertebrates. First, results from Chapters 5 and 6 are used in conjunction with previous research to develop a conceptual model of spatial variability in the environmental characteristics of hyporheic refugia (aim 2, objective 4; aim 3; Figure 7.3); these key characteristics are used to identify sediments with high and low refugial potential (Figure 7.3; Table 7.1). This refugial potential is then considered alongside disturbance characteristics to suggest circumstances under which the hyporheic zone can be expected to act as a refugium (aim 2, objective 4; Table 7.2); the usefulness of this approach is then considered using results from both study rivers. Finally, results from this research project are synthesized with existing knowledge to develop a tool for understanding the behaviour of benthic invertebrates in the hyporheic zone during disturbance events (Figure 7.4).

7.2 Variation in surface flow

Surface flow variability was examined on the River Lathkill (Chapter 5) and the River Glen (Chapter 6) to identify hydrological conditions with the potential to stress benthic invertebrates directly. On the Lathkill, hydrological conditions comprised two elements: a four-month uninterrupted flow recession culminating in partial streambed drying, and a high-magnitude spate (Figure 5.2). The River Glen was more responsive to rainfall inputs and a series of small spates occurred between May and June, followed by a two-month period of low flows, which included habitat contraction and repeated short-duration streambed drying on the East Glen (Figure 6.2). The magnitude of the reductions in discharge are summarised in Figure 7.1.

7.2.1 Flow recession and streambed drying as invertebrate stressors

Flow recession and low flows are common instream disturbances which can have diverse effects on invertebrate community composition as a result of changes in habitat availability, habitat heterogeneity and water quality (Suren *et al*., 2003b; Wood and Armitage, 2004; Dewson *et al*., 2007a). On the Lathkill, flow recession proceeded uninterrupted for >4 months, representing an extended period of moderate flow in a system that regularly experiences hydrological extremes (Figure 5.3). Flow recession was nonetheless potentially stressful for invertebrate fauna, since reductions in depth and width caused widespread declines in submerged habitat availability (Table 5.6). Timing of the exposure of benthic sediments was spatially variable depending on channel morphology (Figure 5.5; Appendix 7) and culminated with drying of two marginal sampling areas in August; flowing surface water therefore remained connected throughout the study area. On the Glen, reductions in discharge, width and depth also decreased habitat availability; these declines were generally of a lower magnitude than on the Lathkill (Table 5.6; Table 6.5) with the notable exception of site 4, where submerged habitat availability was much reduced in July. On both rivers, reductions in width and depth were accompanied by slower flow velocities (Figure 5.4(ii); Figure 6.5(ii)). However, on the Lathkill, fast flowing habitat types were retained at sites 1-4, whilst site 5 remained ponded throughout the flow recession; similarly, fast flowing habitats were retained at Glen sites 1, 2 and 4, whilst velocities dropped sharply between May and June at site 3 and the site then remained largely ponded. In addition, drying of marginal sampling points at Glen site 4 in July and on the Lathkill in August represented the development of a new habitat type and thus increased overall habitat heterogeneity.

Streambed drying represents a period of particularly severe instream conditions for aquatic invertebrates, since the presence of free water is vital to the survival of most taxa (Hynes, 1958; Kownacki, 1985; Boulton, 2003). Whilst streambed drying was only marginal on the Lathkill, discharge data indicated that complete drying occurred at Glen site 4 for several days in late July and again in early September (Figure 6.2).

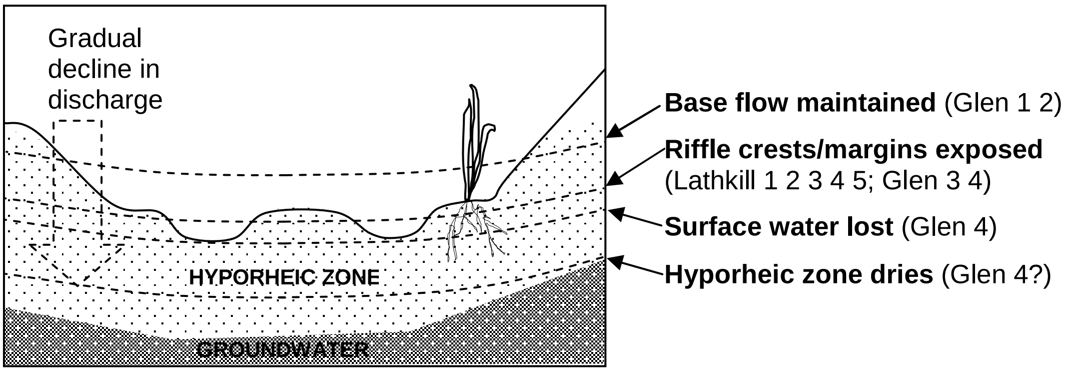


Figure 7.1: Ecologically relevant thresholds during a decline in discharge, indicating conditions experienced on the Rivers Lathkill and Glen (adapted from Boulton, 2003 and Stubbington *et al.*, 2009a). Numbers refers to sites; dashed lines indicate the approximate water surface.

Despite occurring during a period of above-average rainfall and unusually high discharge, the reductions in flow experienced on the Lathkill and the Glen correspond to instream conditions described for low flow disturbances in other systems (often referred to using the general term ‘drought’; Lake, 2000; Boulton, 2003; Lake 2003). Boulton (2003) described a ‘stepped’ model of drought disturbance, in which a decline in discharge is punctuated by ‘critical stages’ for benthic invertebrates, including isolation from riparian vegetation, loss of riffle habitats, and ultimately loss of surface water. This model can be applied to Glen site 4, where base flow was maintained in June, marginal areas were exposed and the extent of riffle habitat reduced in July, and complete streambed drying (with the potential to extend into the hyporheic zone) followed in late July and early September (Figure 7.1). In contrast, whilst the decline in discharge experienced on the Lathkill was considerable, Boulton's (2003) critical stages were only locally transcended and this model is less useful in explaining community response. Instead, the four-month Lathkill flow recession appears to have acted as a 'ramp' disturbance (*sensu* Lake 2000) at some sites (3-5), with disturbance strength and spatial extent increasing steadily over time. At other Lathkill sites (1-2), channel morphology resulted in the decline in flow acting as a 'press' disturbance (*sensu* Lake, 2000) which rose rapidly to a maximum strength which was then maintained. Similarly, at Glen site 3, ponding occurred from June onwards and disturbance strength subsequently increased only slightly.

To summarise, flow recession and low flows were potential stressors of the benthic community primarily due to reductions in habitat availability, which were widespread on the Lathkill but localised on the Glen. Complete streambed drying was restricted to Glen site 4, where environmental conditions would have become particularly severe for benthic invertebrates.

7.2.2 Spates and high flows as invertebrate stressors

Spates are archetypal disturbance events which have pronounced impacts on invertebrate communities primarily through exposure to high shear stress and mobile sediments (Death, 2008). On the Lathkill, flow recession was interrupted by a single, large spate event during which discharge increased twenty four-fold in 25 hours. Whilst this event was not unusual in magnitude (Figure 5.3), the rate at which flow increased was particularly rapid. Individual velocity readings remained above 1.1 m s^{-1} in September as flood waters receded. Higher velocities are inherent in the higher discharges recorded during the flood peak, although difficulties in safely measuring velocities during high flows means that data from comparable situations are not available (Costa *et al*., 2000). The Lathkill spate had marked effects on instream habitats, as evidenced by observations of sediment scour to a depth of 20 cm and damaged vegetation in several sampling areas (e.g. Jowett and Richardson, 1989; Matthaei *et al*., 1999). In contrast, the series of spates on the Glen were low in magnitude, with discharge reaching only one third of that recorded during multiple events in the preceding winter (data not presented). In addition, the Glen's flashy flow regime resulted in the resumption of base flow conditions prior to June sampling, and therefore spate flow velocities remain unknown; there was, however, no evidence of bedload movement or vegetational damage in any sampling area.

Spates are recognised as 'pulse' disturbances (*sensu* Lake, 2000) which can have pronounced impacts on invertebrate communities by initiating displacement and drift (Brittain and Eikeland, 1988; Death, 2008), particularly where sediment is eroded (Strommer and Smock, 1989; Matthaei *et al*., 1999). Whilst flood-mediated entrance into drift has been observed in a wide range of taxa, including species of Chironomidae, Ephemeroptera and Plecoptera (Anderson and Lehmkuhl, 1968), data noting forces required to initiate catastrophic drift for the particular taxa present in the Lathkill are scarce. However, the probability of *Gammarus pulex* occurring in a particular lotic ecosystem has been shown to decline at velocities above 0.7 m s^{-1} (Peeters and Gardeniers, 1998). In addition to the current velocities reached, a change in velocity is an important drift initiator (Anderson and Lehmkuhl, 1968), and drift may be particularly pronounced when a change in discharge follows a long period of stable flow (Irvine, 1985; Perry and Perry, 1986). In the current study, both the rapid rate of flow increase and the preceding flow stability may have increased the impact of the spate on the invertebrate community, particularly on the Lathkill.

In summary, the spate on the Lathkill was a high-magnitude event that reworked surface sediments and had strong potential to alter benthic invertebrate community composition by initiating catastrophic drift in a wide range of taxa. In contrast, the multi-peak event recorded on the Glen had little detectable impact on the instream environment, leaving its ability to impact upon benthic invertebrates in question.

7.3 Changes in surface water quality

In addition to identifying hydrological conditions with the potential to stress benthic invertebrates, potentially detrimental changes in water quality resulting from variation in surface flow were also examined.

7.3.1 Surface water quality during flow recession and low flows

On the Lathkill, flow recession was associated with significant changes in several water chemistry parameters, for example conductivity increased (Figure 5.9), indicating a greater contribution of groundwater to streamflow (Chessman and Robinson, 1987; Harvey *et al*., 1997; Caruso, 2002; Malcolm *et al*., 2004). In contrast, on the Glen, surface water conductivity was particularly low following habitat contraction at site 4 in July, indicating that groundwater inputs were declining. However, conductivity, nutrients and pH all remained moderate in surface water throughout the flow recession and although significant, temporal changes were minor and unlikely to have biotic effects. Surface water POC concentrations were not measured in May or June but increased between July and August on the Lathkill, indicating that resources were sufficient to support the community present; whilst concentrations declined on the Glen, they remained comparable to those on the Lathkill.

One water quality parameter of potential ecological importance was dissolved oxygen. Oxygen availability declined on the Lathkill as flow recession progressed (Figure 5.6), and concentrations were also lower on the Glen under base flow conditions, in particular following habitat contraction at site 4 in July (Figure 6.7). Such reductions may have resulted from reduced turbulence at low flow velocities and/or the increasing dominance of groundwater inputs (Winter *et al*., 2002), whilst the influence of water temperature on oxygen saturation concentrations (Murdoch *et al*., 2000) was not apparent on either river. However, mean % saturation on the Lathkill remained above 90 % in all months (Table 5.7) which is considered ‘excellent’ in terms of ecological health (Gordon *et al*., 2004), with individual readings falling to 76 % at site 1 in August, which is considered ‘good’ (Gordon *et al*., 2004). Such values should not have detrimentally affected the survival of the more oxygen-sensitive taxa (i.e. the Ephemeroptera, Plecoptera and Trichoptera – EPT taxa). Langford (1971), for example, recorded all the Lathkill’s mayflies (i.e. *Baetis* spp., *Serratella ignita*, *Ephemera danica*, *Rithrogena semicolorata* and *Habrophlebia fusca*) and several of the stoneflies (i.e. *Amphinenemoura sulcicollis*, *Isoperla grammatica* and several *Nemoura* and *Leuctra* species) in reaches of a thermally polluted river where oxygen dropped to 71 %; short-term survival of such taxa has also been demonstrated at considerably lower values (Connolly *et al*., 2004).

The Glen is exposed to greater anthropogenic influences on water quality compared with the Lathkill, due to arable, pastoral, urban and low-level industrial land uses within the catchment. These land uses were reflected by lower dissolved oxygen concentrations, higher conductivities, and (on the West Glen) high nutrient concentrations compared with the Lathkill (Table 6.7). Water quality appeared particularly low at site 3, with very high conductivity, high temperature and low oxygen being recorded. During flow recession on the Glen, ponding of site 3 was accompanied by further reduction in oxygen availability, with values falling as low as 66 % saturation in a marginal sampling area, whilst individual readings of 57 % were recorded at contracting site 4 in July. Such values are approaching ‘fair’ (60 %) according to ecological classification schemes (Gordon *et al*., 2004) but may prevent survival of oxygen-sensitive taxa (e.g. Kamler, 1971).

To summarise, reduced oxygen availability was the most biologically-significant change in surface water quality during low flows on both rivers. On the Lathkill, oxygen concentrations nonetheless remained favourable for invertebrate fauna throughout the flow recession, whilst low flows exacerbated water quality issues including DO availability on the Glen, but poor conditions were restricted to ponded site 3.

7.3.2 Surface water quality during spate and high flows

Dissolved oxygen data is not available for the Lathkill in September, however it is probable that increased turbulence, reduced groundwater residence times and low water temperatures would have caused oxygen availability to exceed values recorded in the preceding months (Marmonier and Dole, 1986). In addition, whilst various water chemistry parameters were significantly altered by the increase in flow (e.g. a reduction in conductivity to pre-flow recession values; Table 5.7), all variables remained favourable for biota after the spate. Some potentially relevant variables that may have changed in response to the increase in discharge were not measured during the disturbance, in particular, suspended sediments may have increased due to mobilisation of material from otherwise depositional areas (Milner *et al*., 1981; Wood and Armitage, 1997). Such sediments can have numerous detrimental biotic effects, for example compromising the functioning of respiratory structures (Lemly, 1982) and impairing feeding efficiency in filterer taxa (Aldridge *et al*., 1987). Whilst some recent research has suggested that an increase in flow alone can affect benthic invertebrates, regardless of suspended sediment concentrations or bedload movement (Bond and Downes, 2003), other work has linked dislodgement primarily to substrate stability (Holomuzki and Biggs, 2000). In Lathkill, the increased strength of physical forces (e.g. boundary shear stress, flow velocities, mobile sediments) as discharge increased was very likely a more important determinant of community composition than water quality.

On the Glen, only nitrate showed a consistent response to the May/June spates, with concentrations declining at all sites in June (Table 6.6). Previous studies have noted complex and variable changes in nutrient availability in response to an increase in discharge, with increases in nitrate attributed to a reduction in benthic denitrification (Casey and Farr, 1982) whilst decreases can often be explained by increase in dilution (House and Warwick, 1998) or a reduction in groundwater dominance (Dent *et al.,* 2001). However, nitrate concentrations had returned to May values by July/August (Table 6.6), and such short-term changes in nutrient availability are unlikely to alter instream communities. Therefore, changes in water chemistry with the potential to affect the composition of the benthic invertebrate community were not recorded after the Glen spates.

In summary, the Lathkill spate acted as a resetting event (Junk *et al*., 1989; Lake, 2000) which restored water quality parameters to pre-flow recession levels, whilst high flow events on the Glen had few detectable effects on water quality.

7.4 Benthic invertebrate community composition

The preceding discussion identified several hydrological or hydrologically-mediated conditions with the potential to detrimentally affect the benthic invertebrate community. In this section, observed changes in community composition are discussed and related to these potential stressors.

7.4.1 Effects of streambed drying

Instream conditions would have been harsh for aquatic fauna at Glen site 4 following the loss of surface water (Boulton and Lake, 2008). However, observations regarding the particular effects of streambed drying on the community are confounded by sampling being undertaken between but not during dry phases. Nonetheless, considerable reductions in invertebrate abundance and diversity at site 4 between July and August, and further reductions in September are consistent with previous research highlighting the severe impacts of complete drying on instream communities (Figure 6.15; Kownacki, 1985; Wright and Berrie, 1987; Smock *et al.*, 1994; Fritz and Dodds, 2004; Wood and Armitage, 2004). Whilst the dry phase persisted for a shorter duration than reported by many studies (days rather than months), invertebrates are unlikely to survive the physiological stress of desiccation for more than a few hours or days, due to the vital importance of water as the major component of body tissues and as the solution in which most metabolic reactions occur. The amphipod *Gammarus pulex*, for example, was observed to persist in moist conditions under large substratum particles shortly after surface water was lost from the ephemeral headwaters of the Lathkill (a habitat not considered in the current study) but was absent 11 days later (Stubbington *et al*. 2009b). However, whilst benthic invertebrates in temperate streams are typically not resistant to drying disturbance (i.e. they do not survive), they are often highly resilient (i.e. they recover quickly), particularly where habitats are longitudinally connected to perennial waters (Ledger and Hildrew, 2001; Fritz and Dodds, 2004). The benthic community present at site 4 in August and September is therefore likely to be dominated by recolonists arriving from drying refugia following the resumption of surface flow.

7.4.2 Effects of flow recession – invertebrate densities

The condition identified as the primary potential stressor during low and declining flow conditions on the Lathkill was the reduction in submerged benthic habitat availability (Table 5.6). Previous work in both intermittent and perennial streams has attributed the occurrence of peak invertebrate densities during a decline in discharge to the concentration of a stable population into a smaller area of submerged benthic sediments (Covich *et al*., 2003; Fritz and Dodds, 2004; Dewson *et al*., 2007b). In contrast, decreases in abundance reported by other studies have been attributed to reductions in inhabitable space, changes in biotic interactions and/or changes in resource availability (McIntosh *et al*., 2002; Kinzie *et al*., 2006; Dewson *et al*., 2007a). On the Lathkill, there was no significant change in total invertebrate abundance during the flow recession (Table 5.10), whilst changes on the Glen between June and August largely reflected seasonal variation in insect taxa (Figure 6.8). However, stable total invertebrate abundance on the Lathkill disguised a significant threefold increase in the abundance of the dominant benthic taxon, the amphipod *Gammarus pulex*, between May and August (Figure 5.15(i)), whilst the same taxon was particularly abundant following habitat contraction at Glen site 4 in July.

Whilst some previous studies have noted fatal stranding of *Gammarus* species including *G. pulex* following exposure of surface sediments (Extence, 1981; Ayers *et al*., 1998; Stubbington *et al*., 2009b), the species is typically highly mobile in upstream, downstream and lateral directions (Hughes, 1970; Pearson and Jones, 1987; Elser, 2001) and many individuals should therefore be capable of following a slowly receding water line. Consequently, it is probable that the recorded increases in *G. pulex* abundance primarily reflect the concentration of a numerically stable population into a contracting habitat area, although favourable conditions in the remaining submerged habitat may have allowed concurrent population expansion. Evidence supporting this suggestion comes from two sources. Firstly, research in other streams has found no significant differences in benthic *G. pulex* abundance between months, with particular stability being observed between spring and October (Macan and Mackereth, 1957); other studies have recorded differences between months, including some increase in spring, but population stability appears to be the norm during the summer months (Mortensen, 1982; Graça *et al*., 1994; Wood *et al*., 2010). Secondly, sampling undertaken at Lathkill site 5 in 2009 confirmed that *G. pulex* did not experience significant fluctuations in population densities between May and September when habitat availability remained constant (section 5.8; Table 5.9).

That the *G. pulex* population experienced concentration and not a numerical increase in abundance is also supported by the contrasting patterns observed at Lathkill sites with different channel morphology and therefore different patterns of drying (Figure 5.5). At sites 1 and 2, small declines in depth were sufficient to expose considerable areas of mid-channel benthic sediments in June and further reductions in habitat availability were minimal. Accordingly, May-June increases in *G. pulex* densities were followed by population stability at these sites (Figure 5.15). In contrast, at sites 3-5, the exposure of marginal areas was a gradual process that culminated in the loss of surface water from two sampling points in August. At these sites, *G. pulex* densities tended to increase gradually over the four months, although peak abundance was not recorded in August at site 3 due to the absence of *G. pulex* from a dry sampling area. This sudden loss of *G. pulex* from a sampling area where abundance had previously been rising and where densities of >4000 m^{-2} had been recorded the previous month indicated that the critical threshold (*sensu* Boulton, 2003) at which conditions become unfavourable for a taxon as flow declines was reached for *G. pulex* shortly before the loss of surface water.

7.4.3 Effect of flow recession - community diversity

The four-month flow recession on the Lathkill can be viewed either as a prolonged period without disturbance (at least in the submerged habitat that remained), or alternatively as a single high-magnitude ramp disturbance during which habitat availability became increasingly restricted. Excluding potentially seasonal changes in insect taxa, significant increases in abundance were restricted to a single highly competitive taxon, *G. pulex* (Table 5.13). Similar increases were seen following habitat contraction on the Glen, but were not significant (Table 6.11).

Previous work has also noted significant increases in abundant taxa following a flow reduction, but this has not been associated with reductions in community diversity or dominance (Dewson *et al*., 2007b, although this study only considered month-long reduced-flow period). In contrast, on the Lathkill, the increase in *G. pulex* abundance was accompanied by an increase in the proportion of the community accounted for by this taxon and consequently, a significant reduction in community diversity and an increase in dominance (Figure 5.14). However, a key component of a diversity index is taxonomic richness, which, in contrast to previous studies (Rader and Belish, 1999; McIntosh *et al*., 2002; Dewson *et al*., 2007a) did not decline during the Lathkill flow recession (Figure 5.14(ii)); indeed, when seasonal EPT taxa were excluded, mean taxon richness increased slightly between May and August. This could be attributed to water quality remaining favourable and habitat heterogeneity increasing rather than decreasing (as reported by McIntosh *et al*., 2002; Dewson *et al*., 2007a). In particular, drying of marginal benthic sediments increased taxonomic richness in both rivers by creating damp-margin microhabitats capable of supporting semi-aquatic Coleoptera.

Therefore, like much previous research in lotic ecosystems (Reice, 1981; Death and Winterbourn, 1995; reviewed by Death, 2010), the current study contradicted the dominant theory of community diversity, the intermediate disturbance hypothesis, which states that community diversity will be reduced where environments remain undisturbed, due to competitive exclusion by dominant taxa (also see Hardin, 1960; Grime, 1973; Connell, 1978; Ward and Stanford, 1983). Instead, results from the Lathkill indicated that less competitive taxa are not excluded at low levels of disturbance, and in addition, new taxa may be able to join the community in marginal microhabitats; however, these rarer taxa may account for a small proportion of the total community.

On the Glen, low flows between June and August were associated with localised declines in surface water quality, in particular lower dissolved oxygen availability, with sites 3 and 4 being most adversely affected (Figure 6.7). However, differences in invertebrate diversity and richness appeared to be related to spatial variability in habitat heterogeneity rather than temporal variation in water quality, with fewer taxa recorded at site 3 compared with other sites, including site 4 (Table 6.9). Site 3 was slow flowing, heavily shaded and lacked submerged macrophyte beds, thus precluding rheophilic taxa associated with macrophytic habitats, such as Simuliidae (Harrod, 1964; Horne *et al*., 1992), whilst a scarcity of exposed benthic sediments limited habitat suitability for semi-aquatic taxa such as *Helophorus brevipalpis* (Sadler and Bell, 2002).

To summarise, on the Lathkill, flow recession and habitat contraction were associated with a significant increase in *G. pulex* abundance which caused community diversity to decline, although taxon richness of aquatic fauna was unaffected. Similarly, on the Glen, habitat contraction was associated with localised concentration of benthic invertebrates, whilst differences in richness and diversity were more strongly related to site-specific conditions than flow-related changes. On both rivers, marginal drying increased taxonomic diversity in semi-aquatic taxa, due to the provision of a new habitat type.

7.4.4 Effects of high flows

According to the harsh-benign hypothesis (Menge and Sutherland, 1976), *harsh* conditions indicate an environment dominated by an extreme abiotic condition (Townsend *et al*., 2003; Death, 2010). The high-magnitude Lathkill spate would be expected to constitute such an abiotic condition, capable of overshadowing other biotic and environmental influences on community structure. In contrast, the series of high flow events on the Glen were of a lower magnitude and could therefore be expected to have less pronounced effects on an invertebrate community adapted to frequent events of the observed magnitude and duration (Lytle and Poff, 2004).

Following the Lathkill spate, substantial reductions in total invertebrate abundance (mean -62 %) were recorded in the benthic sediments (Figure 5.14(i)). Although considerable, these declines were not particularly severe; Fritz and Dodds (2004), for example, found benthic population densities to be reduced by >99 % after a >50 year recurrence interval flood, whilst Olsen and Townsend (2005) noted reductions of 89 % following an event with a 1.5 year estimated return period. Such reductions reflect displacement of invertebrates during conditions of high shear stress, in addition to crushing by mobilised bed sediments and indirectly through loss of food resources (Poff and Ward, 1989; Bond and Downes, 2003; Death, 2008). However, in the Lathkill, the general pattern of reduced abundances was not without exception. Species of Chironomidae, for example, increased in abundance at three sites, both *P. felina* and the Oligochaeta became more common in some areas of four sites, and various EPT taxa also increased in abundance locally. In all cases, these increases can potentially be explained by animals being displaced from deeper hyporheic sediments (Dole-Olivier *et al*., 1997): *P. felina* is common in groundwater dominated karst rivers (Rada and Puljas, 2010) and is morphologically suited to an interstitial existence; oligochaetes are also morphologically flexible and are common members of the permanent hyporheos; and many insect taxa use the hyporheic zone as a nursery for young instars (Jacobi and Cary, 1996).

In the Glen, whilst total invertebrate abundance declined considerably in June (after the spates; Figure 6.15(i)) this largely reflected a reduction in chironomid abundance (Table 6.11); such a decline may partly reflect seasonal emergence of adult life stages as well as disguising potentially contrasting responses of individual taxa. Such factors may also be partly responsible for a considerable decline in the abundance of Simuliidae larvae on the West Glen between May and June; however, this taxon exploits positions exposed to the flow in order to filter feed and as such is prone to displacement (Maitland and Penney, 1967). This limited impact of the Glen spate on the benthic community was unsurprising considering the low disturbance magnitude and lack of observed effects on instream habitats.

Through the displacement of large proportions of the invertebrate community, spates are recognised as events that reset successional trajectories (Fisher, 1983; Power *et al*., 1988). Such events can also potentially restore community diversity due to disproportionate impacts on competitive taxa which are not resistant to disturbance (Death, 2008). In the Lathkill, reductions in abundance were particularly severe in the most dominant taxon, *G. pulex* (Figure 5.15(i)) whilst mean taxon richness decreased only slightly, and the spate was therefore associated with an increase in Simpson's diversity (Figure 5.14); such results provide equivocal support for the intermediate disturbance hypothesis, which predicts that both richness and diversity will decline (Grime, 1973; Connell, 1978; Ward and Stanford, 1983). On the Glen, although the spates were only linked to significant reductions in the most dominant taxon (the Chironomidae) and had little detected impact on taxonomic richness, dominance and diversity indices were very similar before and after the spates, emphasizing the low-magnitude of these disturbances (Figure 6.15).

In summary, spates caused reductions in invertebrate abundance on both rivers whilst declines in taxon richness were very minor. On the Lathkill, disproportionate reductions in the dominant *G. pulex* restored community diversity, whilst declines in the Chironomidae on the Glen spates had no such effects. However, the low level of taxonomic resolution to which some groups (particularly the family Chironomidae and the subclass Oligochaeta) were identified in the current study limits the extent to which conclusions can be drawn regarding community richness and diversity. Langton and Casas (1998), for example, found that whilst several species of Chironomidae were eliminated during a period of high discharge, there was an overall increase in taxon richness in this family. Identification would need to be conducted to species-level to determine whether patterns observed at the group level are representative of all species.

7.5 Hydrologically-mediated changes in biotic stressors

In addition to the identified hydrological and related environmental stresses, some described compositional changes in the invertebrate community had the potential to increase biotic pressures in the benthic sediments. External (non-invertebrate) biotic stresses may also have been altered by hydrological variability and require consideration. According to the harsh-benign hypothesis (Menge and Sutherland, 1976; Peckarsky, 1983), at the benign end of an environmental gradient, biotic factors such as competition and predation increase in importance as determinants of community composition (Lancaster, 1996; Death, 2010). In the current study, flow recession and low flows constituted relatively benign hydrological conditions and as such biotic factors may have become increasingly influential over time; the following discussion is therefore focussed on these conditions.

7.5.1 Invertebrate causes of increased biotic interactions

Habitat contraction resulted in *G. pulex* population densities reaching high densities on the Lathkill, with mean densities of 2321 m^{-2} and peak densities of 6480 m^{-2} recorded in August (Figure 5.15(i)). These figures exceed those reported in many other studies, for example average densities of 200 m^{-2} were reported from two small streams in the English Lake District (Macan and Mackereth, 1957), and similarly Crane (1994) recorded estimated mean densities of 149 m^{-2} in a Yorkshire stream and 267 m^{-2} in an Oxfordshire stream. Other studies have, however, noted comparable densities to those reported here, for example peak densities of 5500 m^{-2} in a small Danish stream (Mortensen, 1982), and mean densities of 6886 m^{-2} in the River Darenth in Kent (Crane, 1994); hydrological conditions are not known for these studies. Nonetheless, it is probable that the observed threefold increase in the high-density *G. pulex* population in the Lathkill was sufficient to cause a range of biotic interactions to intensify in the benthic sediments. In contrast, whilst habitat contraction at Glen site 4 in July was accompanied by *G. pulex* densities fourfold higher than in any other month, peak densities were only 501 m^{-2}; such densities are considered unlikely to cause biotic interactions to increase significantly.

G. pulex is a highly competitive species and is able to outcompete a diverse range of other taxa for space and food resources (e.g. Hynes, 1954; Graça *et al*., 1993). Gammarids including *G. pulex* have traditionally been considered herbivores and assigned to the 'shredder' functional feeding group, which primarily consumes coarse particulate organic matter (Willoughby and Sutcliffe, 1976; Cummins and Klug, 1979). However, it is now known that the species exhibits dietary plasticity and has a considerable predatory component in its feeding strategy where resources permit (Moore, 1975 and references therein; Savage, 1996; MacNeil *et al*., 1997, 1999; Kelly *et al*, 2002). In addition, cannibalism has been demonstrated as common in *G. pulex*, with smaller individuals and those at moult the most targeted prey (Jenio, 1979; Dick, 1995; McGrath *et al*., 2007; Lewis *et al*., 2010).

The flatworm *Polycelis felina* (Turbellaria: Planariidae) was common at Lathkill sites 1 and 2 and increased sharply in abundance between May and June, with mean densities of 1740 m^{-2} and peak densities of >3000 m^{-2} recorded at site 1 in June (Figure 5.15(ii)). Abundance then remained high at both sites until August, suggesting that, as reported for *G. pulex*, the apparent increase in numbers reflected concentration of a stable population into a contracting habitat area. *P. felina* is a predatory taxon and previous studies have linked an increase in its abundance to declines in species of benthic feeding mayflies (Macan, 1963); it is also known to predate *Gammarus* spp. (Lock and Reynoldson, 1976; Reynoldson, 1981), whilst field evidence of cannibalism is lacking (Davies and Reynoldson, 1971). *P. felina's* predation technique involves laying traps of mucus strings *over* substrate particles, but not *under* them (Macan, 1963); high abundance of this taxon may therefore have exacerbated the proposed increase in biotic pressures, particularly in the surficial sediments.

7.5.2 Non-invertebrate biotic stressors

The decline in total submerged habitat forced benthic invertebrates into a smaller space, and previous research has suggested that habitat suitability in a contracting submerged area may be further limited by requirements that reduce exposure to predation, for example a minimum water depth (Power, 1984). Predators including brown trout (*Salmo trutta*: Salmonidae) and dippers (*Cinclus cinclus*: Passeriformes: Cinclidae) are common in the Lathkill Dale (P. Bowler, pers. comm.), and both consume a range of invertebrates, including *G. pulex* (Elliott, 1967; Ormerod *et al*., 1986; MacNeil *et al*., 1999). Previous work has suggested that low water depth improves feeding efficiency in dippers (D'Amico *et al*., 2000), whilst lower flow velocities improve habitat suitability for trout, although this may be counteracted by reductions in depth (Heggenes, 1996). The decline in water depth recorded on the Lathkill therefore potentially increased exposure to a range of predation pressures. However, the overall pattern of an increase in *G. pulex* abundance as depth declined was only halted by the complete loss of surface water (observed at single sampling points at sites 3 and 4), indicating that lateral migration into deeper waters only occurred for physiological reasons, not to reduce predation pressures. The range of invertebrate predators present on the Glen is not known.

7.6 The hyporheic zone as an invertebrate refugium

Taxa or individuals that are unable to tolerate an adverse condition that develops in the surface stream must instead try to avoid it, for example by moving into refugial habitats that promote survival (Boulton, 2003; Humphries and Baldwin, 2003). One such potential refugium is the hyporheic zone (Williams and Hynes, 1974; Robertson and Wood, 2010), and the central focus of work conducted on the Lathkill and Glen was to determine if conditions identified as potential stressors of the benthic community triggered active vertical migrations into the hyporheic zone. First, the factors controlling the ability of the hyporheic sediments to act as a habitat for benthic invertebrates are considered.

7.6.1 Environmental factors affecting functioning of the hyporheic refugium

The physical environment of the hyporheic zone is central in determining the composition of the invertebrate community it can support, and to function as an effective refugium, certain environmental criteria must be fulfilled. Reference to the literature indicates that three interrelated environmental variables are commonly cited as responsible where the hyporheic zone is not actively used as a refugium: hydrologic exchange, dissolved oxygen availability and sediment composition. The known effects of and relationships between these individual factors will first be considered, and then will be used to examine the potential of the Lathkill and Glen hyporheic zones to act as refugia.

1. Hydrologic exchange

The hyporheic zone contains both downwelling water from the surface stream and upwelling inputs from the groundwater aquifer (Jones and Holmes, 1996; Brunke and Gosner, 1997), and the direction and strength of this hydrologic exchange influences water chemistry, temperature regime, and the supply of oxygen, nutrients and organic matter to the hyporheic zone (Boulton, 1983; Grimm and Fisher, 1984; Brunke and Gosner, 1997). As such, previous studies have noted hydrologic exchange as an important determinant of the community resident in the hyporheic zone (Olsen and Townsend, 2003; Davy-Bowker *et al*., 2006), with downwelling water supporting a greater abundance and diversity of predominantly benthic organisms (Creuzé des Châtelliers and Reygrobellet, 1990; Dole-Olivier and Marmonier, 1992a; Boulton and Stanley, 1995). Few studies suggest particular features of these up- or downwelling zones as being of overriding importance in determining community composition, with a combination of factors instead being apparently responsible Davy-Bowker *et al*., 2006). Firstly, downwelling water originates from and has similar characteristics (oxygen, temperature, nutrients, conductivity) to the surface stream, whilst groundwater-dominated upwelling inputs have distinct chemical characteristics (Datry *et al*., 2007). Secondly, downwelling water provides inputs of organic matter food resources, whilst upwelling water is typically low in organic matter, reducing the ability of the zone to support epigean taxa (Datry *et al*., 2005). In addition, the direction of water movement in downwelling zones facilitates both active and passive transport of benthic organisms into deeper sediments, whilst upwelling water may act as a barrier to migration, particularly at high discharge (Marmonier and Creuzé des Châtelliers, 1991; Dole-Olivier *et al*., 1997).

2. Dissolved oxygen and water quality

Low dissolved oxygen availability may prevent survival of sensitive taxa in hyporheic sediments, and interstitial oxygen concentrations have been shown to be positively correlated with invertebrate community metrics including abundance (Williams and Hynes, 1974; Boulton *et al*., 1997; Franken *et al.*, 2001), biomass (Strommer and Smock, 1989), and species richness, including the number of benthic taxa present (Boulton *et al*., 1997; Franken *et al.*, 2001). In contrast, other studies have found weak relationships between DO concentrations and hyporheic community metrics (Strayer *et al*., 1997; Malard and Hervant, 1999), particularly in well-oxygenated sediments; this indicates that relationships are only apparent where a strong gradient exists (Olsen and Townsend, 2003) and well-oxygenated sediments should be able to support a range of benthic refugees.

3. Sediment composition

Sediment composition is an important determinant of the hyporheic community as it dictates porosity, which directly influences the availability of interstitial habitat and the strength of hydrologic exchange (Brunke and Gosner, 1997; Wood and Armitage, 1999). The proportion of fine sediment has been cited as a particularly influential sediment characteristic, with several studies demonstrating negative relationships with community metrics (Richards and Bacon, 1994; Olsen and Townsend, 2003; Weigelhofer and Waringer, 2003); the precise definition of 'fine' sediment in these studies varied between 150 µm (Richards and Bacon, 1994) and 2 mm (Weigelhofer and Waringer, 2003). Fine sediments may also reduce the ability of the hyporheic zone to act as a refugium during disturbances at the hydrological extremes: during spates, fine sediments are prone to bedload movement, so invertebrates may be displaced (Palmer *et al*., 1992; Dole-Olivier *et al*., 1997; Olsen and Townsend, 2005), whilst following streambed drying, fine sediments may dry to form a crust at the sediment surface which separates the saturated hyporheic zone from aerating inputs of atmospheric oxygen (Gagneur and Chaoui-Boudghane, 1991; Belaidi *et al*., 2004).

4. Relationships between hydrologic exchange, sediment and oxygen

The three factors discussed above interact to determine the refugial capacity of the hyporheic zone (Vervier *et al*., 1992; Robertson and Wood, 2010; Figure 7.2). Hydrologic exchange can influence sediment composition, and during conditions of baseflow, upwelling groundwater typically reduces siltation whilst downwelling groundwater can introduce fine sediment into interstices (Brunke and Gosner, 1997). In return, sediment composition influences hydrologic exchange, with a high proportion of fine sediment reducing exchange strength whilst coarse sediments promote free movement of water (Brunke, 1999; Hancock, 2002). As a consequence, fine sediments receive a reduced supply of dissolved oxygen whilst coarse sediments may be better oxygenated (Wu, 2000), although this is dependent on hyporheic flow velocities (Findlay, 1995). The direction of exchange also influences oxygen content,

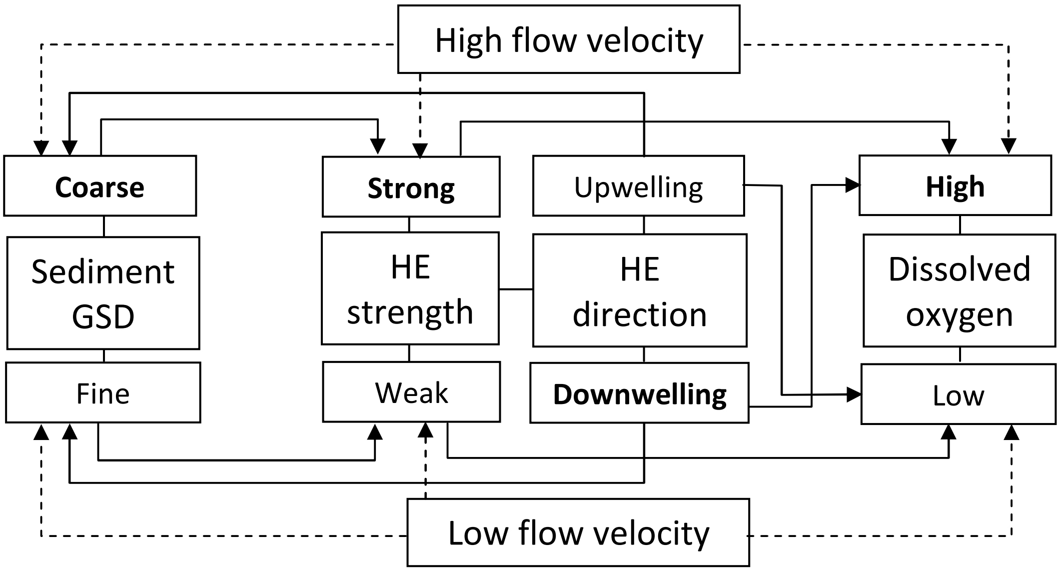


Figure 7.2: Interactions between sediment grain size distribution (GSD), hydrologic exchange (HE), dissolved oxygen concentrations and flow velocity in the hyporheic zone. Solid lines = spatial relationships; dashed lines = temporally variable relationships; bold text = high refugial capacity.

with downwelling water typically being well oxygenated, whilst upwelling water may have low concentrations if dominated by groundwater (Franken *et al.*, 2001; Youngson *et al.*, 2004).

Therefore, the ability of the hyporheic zone to act as a refugium is a trade-off, with no one set of conditions resulting in a 'perfect' refugial habitat: downwelling water has many physicochemical advantages for refugees but interstitial spaces may become clogged, whilst upwelling water clears interstices but may be less appropriate both chemically and hydrologically. Features of hyporheic sediments with generally 'high' and 'low' potential to act as a refugium are summarised in Figure 7.3, with reference to low flow conditions.

7.6.2 Determination of refugial potential

In accordance with the preceding discussion, the direction and strength of hydrologic exchange, the availability of oxygen and the proportion of fine sediment (in McNeil bulk sediment samples) were used to assess the potential of the hyporheic zone to act as a refugium, i.e. the *refugial potential*, of each site on the Lathkill and the Glen (Table 7.1). Robertson and Wood (2010) used a similar term (*refugial effectiveness*) to describe the hyporheic zone's ability to function as a refugium; the word *potential* is preferred here as no assumption of refugium use is inferred.

In addition to the three factors considered, several other variables have been demonstrated as important influences on hyporheic community composition and refugial potential, including sediment volume, connectivity with benthic habitats, bed stability (Vervier *et al*., 1992; Robertson and Wood, 2010) and the availability of particulate organic carbon (POC; Datry *et al*., 2005). Sediment volume has not been included in the current analysis since all sites were located in alluvial reaches where the extent of the sediments was sufficient to permit refugium use, whilst connectivity and bed stability can be inferred from the proportion of fine sediments. POC has also been excluded as hyporheic concentrations were generally high compared with those reported in other gravel-bed streams (Brunke and Gosner, 1999) and organic food resource availability should not have limited refugial potential at any site. Other physicochemical water parameters (e.g. temperature, nutrient concentrations) tend to have weak relationships with community composition in temperate environments (Williams and Hynes, 1974; but see Boulton, 1989) and have not been considered.

Calculation of refugial potential from the selected variables is displayed in Table 7.1. Negative scores indicated that one or more factors limited the potential of the hyporheic zone to act as a refugium, whilst positive scores increased as the subsurface sediments became more suitable for inhabitation by benthic invertebrates.

The highest positive score calculated (4) occurred at Lathkill site 3, reflecting a combination of strongly downwelling water and high oxygen concentrations; this site did not achieve the highest refugial potential (6) due to a moderate proportion of fine sediments (arbitrarily defined as those <1 mm, an intermediate value in comparison with previous studies (Richards and Bacon, 1994; Weigelhofer and Waringer, 2003)). Similarly, Lathkill sites 2, 4 and 5 achieved positive scores due to both downwelling water and high oxygen concentrations, but again refugial potential

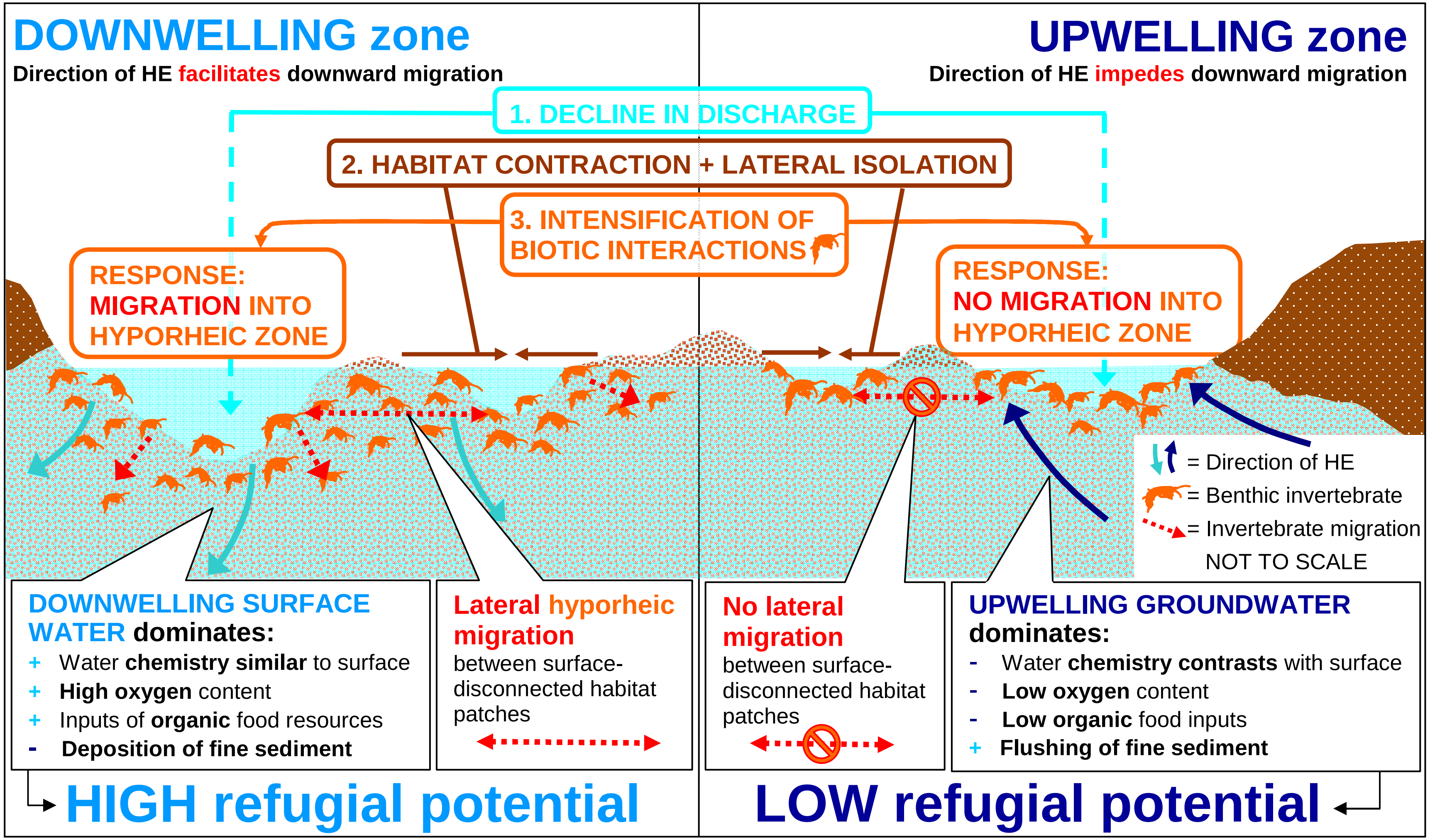


Figure 7.3: Conceptual model of spatial variability in the refugial capacity of the hyporheic zone during low flow conditions, indicating differences between downwelling and upwelling zones. HE = hydrologic exchange. For an explanation of 'refugial potential', see Table 7.1. +/- = factor increases or decreases refugial potential. Channel cross-sectional profile is loosely based on bed morphology at site 1 on the River Lathkill.

Table 7.1: Effect of environmental parameters on the refugial potential of the hyporheic zone at sites on the River Lathkill and River Glen

River	Site	1. Hydrologic exchange*	2. Dissolved oxygen**	3. Fine sediment§	§§Refugial potential (1 + 2 + 3) =
Lathkill	1	↓↓	↔	↑	-1
	2	↔	↑↑	↔	2
	3	↑↑	↑↑	↔	4
	4	↑↑	↑↑	↓	3
	5	↑↑	↑	↓	2
Glen	1	↔	↑↑	↓↓	0
	2	↔	↑	↓	0
	3	↓	↑	↓↓	-2
	4	↑↑	↓↓	↓	-1

Notes:

Effect on refugial potential: ↑ = increase; ↓ = decrease; ↔ = no effect

***Hydrologic exchange (dominant direction and approximate strength)**
↓↓strong upwelling; ↓upwelling; ↔up & downwelling; ↑downwelling; ↑↑ strong downwelling

****Hyporheic dissolved oxygen (mean)**
↓↓ <3 mg L^{-1}; ↓ 3–4 mg L^{-1}; ↔ 4–5 mg L^{-1}; ↑ 5–6 mg L^{-1}; ↑↑ >6 mg L^{-1}

§Fine sediment (proportion of sediments <1 mm)
↓↓clay present & >40 % FS; ↓30-40 % FS; ↔20-30 % FS; ↑<20 % FS

§§Refugial potential calculation: ↓↓ = -2; ↓ = -1; ↔ = 0; ↑ = +1; ↑↑ = +2

was tempered by moderate to high proportions of fine sediment. In contrast, Lathkill site 1 had a negative score despite particularly low amounts of fine sediments, since refugial potential was compromised by strongly upwelling water and associated low oxygen concentrations.

On the Glen, no site had a positive refugial potential score. This was due in part to a high proportion of fine sediments at all sites, particularly at sites 1 and 3, where clay layers were observed in some samples. In addition, the typical relationship between hydrologic exchange and oxygen availability (downwelling surface water = higher hyporheic oxygen; upwelling groundwater = lower hyporheic oxygen) was not observed, and the high refugial potential conferred by strongly downwelling water at site 4 was compromised by low mean oxygen availability (largely due to several values approaching zero in August and September in the aftermath of drying events). Equally, the benefits of moderate-high mean oxygen concentrations at sites 1-3 were partly offset by upwelling water, particularly at site 3.

7.6.3 Temporal variability in refugial potential

Despite the relative stability of the hyporheic zone compared with the benthic sediments, this remains a temporally variable habitat and all parameters used to determine refugial potential may have been altered by changing hydrological conditions on both rivers (Matthaei *et al*., 1999; Wondzell and Swanson, 1999; Hancock, 2006; Olsen *et al*., 2010). Firstly, the direction as well as the strength of hydrologic exchange can temporarily change during high flows (Baker and Vervier, 2004; Malcolm *et al*., 2004), and upwelling groundwater may therefore have prevented a refugium effect in typically downwelling areas (Dole-Olivier *et al*., 1997). In addition, the strength of hydrologic exchange is likely to have increased in upwelling zones (in particular Lathkill site 1; section 5.10.2) in response to increased inputs from groundwater springs. Secondly, spate events may improve hyporheic oxygen availability through increased turbulence, shorter residence times and a reduction in groundwater dominance (Brunke and Gosner, 1997), whilst low flows and in particular streambed drying can have the opposite effect, reducing the supply of oxygen, nutrients and food resources, and allowing metabolic waste products to accumulate in interstices (Dewson *et al*., 2007a); reduced mean oxygen concentrations at Glen site 4 particularly reflected low values recorded after drying events, and concentrations are likely to have been further reduced during the dry phase. Regarding sediment composition, fine sediments may be deposited in interstices during a decline in discharge (Holmes *et al*., 1994; Kondolf and Wilcock, 1996; Wood and Armitage, 1999), as occurred during the Lathkill flow recession. Faster interstitial velocities during spate events typically remove excess fine sediments (Schälchli, 1992; Sophocleous, 2002; McKenzie-Smith *et al*., 2006), but can equally deposit fine material in some habitat patches (Matthaei *et al*., 1999; Olsen *et al*., 2010). On the Lathkill, fine sediment and POC concentrations were generally lower in September (Figure 5.8), indicating that material deposited during the preceding flow recession had been flushed out.

7.6.4 Expected refugium use based on refugial potential and disturbance characteristics

From the calculation of refugial potential, it was expected that, during adverse conditions in the surface sediments, benthic invertebrates would migrate into the hyporheic zone at sites 2-5 on the Lathkill, whilst such behaviour was unlikely at Lathkill site 1 and Glen sites 3 and 4, and predictions for Glen sites 1 and 2 were less certain (Table 7.1). However, the spatial heterogeneity of instream habitats renders it unlikely that these factors alone would dictate hyporheic refugium use in all areas (Lancaster and Belyea, 1997; Lancaster, 2008) during a disturbance. Indeed, disturbance-related parameters are also recognised as important determinants of refugium use (Perry and Perry, 1986), in part due to the described effects on the hyporheic environment, but disturbance characteristics may also prevent migrations into the hyporheic zone at sites where refugial potential remains high. Rapid onset of high-magnitude spate events, for example, may allow insufficient time for invertebrates to respond by migrating downwards (Imbert and Perry, 1999; Gayraud *et al*., 2000), with entrance into the drift being more likely (Brittain and Eikeland, 1988). Equally, Lancaster (2000) and Boulton *et al*. (2004) have both suggested that the failure of the hyporheic zone to function as a refugium during experimental spates resulted from increases in discharge being of too low a magnitude to elicit a behavioural response. At the other extreme, the onset of a streambed drying disturbance is generally slow compared with a spate event (Lake, 2000), providing sufficient time for invertebrates to migrate into the hyporheic zone. However, high magnitude drying disturbances that include the loss of free water from the hyporheic zone severely compromise refugial integrity (Gagneur and Chaoui-Boudghane, 1991; Boulton and Stanley, 1995), as may have occurred at site 4 on the Glen. Such disturbance-related parameters were therefore considered in conjunction with calculated refugial potential to predict the occurrence of refugium use in response to disturbance events affecting both rivers (Table 7.2).

Consideration of the characteristics of the various disturbing forces resulted in some alteration of expected refugium use. On the Lathkill, the rapid onset and high magnitude of the spate was expected to prevent refugium use at all sites, even those

Table 7.2: Expectation of refugium use from refugial potential* and disturbance characteristics

River	Site	Refugial potential	Adverse condition	Disturbance characteristics	Refugium use expected?
Lathkill	1	NEGATIVE	Spate	Rapid onset, high magnitude	No
			Biotic	Slow onset, moderate magnitude	No
	2, 3, 4, 5	POSITIVE	Spate	Rapid onset, high magnitude	No
			Biotic	Slow onset, moderate magnitude	Yes
	3, 4	POSITIVE	Marginal drying	Slow onset Highly localised	No
Glen	1, 2	0	Spate	Rapid onset, Low magnitude	No
	3, 4	NEGATIVE	Spate	Rapid onset Low magnitude	No
	3	NEGATIVE	Ponding	Slow onset	No
	4	NEGATIVE	Biotic	Slow onset, low magnitude	No
			Drying	Slow onset High magnitude	Yes

***see Table 7.1 for calculation of refugial potential.**

with high-refugial potential (Table 7.2). The increase in the abundance of *G. pulex* was considered a biotic disturbance of 'moderate' magnitude, since the disturbance significantly altered benthic community composition but did not have the dramatic impact of the spate. The onset of this biotic disturbance was sufficiently slow for a behavioural response, i.e. migration into hyporheic sediments. However, the moderate disturbance magnitude meant that whilst conditions may have been preferable to the benthic zone in high-refugial capacity hyporheic sediments, the low refugial capacity of site 1 was predicted to result in benthic invertebrates remaining near the sediment surface. Streambed drying was very localised on the Lathkill and refugium use was not expected as lateral connections with submerged benthic habitats were retained. On the Glen, the low magnitude of the spate was not expected to be of sufficient magnitude to trigger vertical migrations into the hyporheic zone, which was at best of moderate refugial capacity. Similarly, low refugial capacity was likely to outweigh the negative effects of ponding at site 3 and the low magnitude increase in biotic interactions at site 4. However, the loss of surface water represents a high magnitude disturbance for aquatic invertebrates, and the occurrence of any drying event was expected to outweigh the low refugial capacity of the hyporheic zone at site 4, resulting in use of the hyporheic zone refugium, particularly since the slow onset of such disturbance events allow time for a behavioural response (Table 7.2).

Next, use of the hyporheic zone will be considered during each of the main disturbance types (low flows including habitat contraction and an increase in biotic interactions; spates; streambed drying), and these observations of refugium use will subsequently be compared to the predictions outlined above.

7.7 Use of the hyporheic zone refugium during low flows

Few previous studies have considered use of the hyporheic refugium during low flow conditions (James *et al*., 2008; James and Suren, 2009), and only one previous study has included a (two month) period of gradually declining flow (Stubbington *et al*., 2009a; Wood *et al*., 2010). Of these studies, none has directly linked a reduction in flow to increased abundance of benthic taxa in the hyporheic zone, and whilst changes in submerged habitat area have not always been determined (Stubbington *et al*., 2009a; Wood *et al*., 2010) or have remained unchanged (James and Suren, 2009), James *et al*. (2008) noted changes in habitat availability of a similar magnitude to those observed in the Lathkill. This previous lack of benthic invertebrate migrations during low flows has been attributed to conditions remaining favourable in the benthic sediments, or at least preferable to conditions in the hyporheic zone (James *et al*., 2008). However, in addition to its refugial role during environmental disturbances, the hyporheic zone has also been proposed as a refugium from high-risk biotic conditions in the surface sediments (Marmonier and Creuzé des Châtelliers, 1991; Dole-Olivier et al., 1997), and protection of vulnerable life stages is the assumed rationale for use of the hyporheic zone as a nursery for early instars (Puig *et al*., 1990; Jacobi and Cary, 1996).

7.7.1 Potential triggers of refugium use

Flow recession on both the Lathkill and the Glen was accompanied by reductions in submerged habitat availability (Table 5.6; Table 6.5). These reductions were widespread on the Lathkill, were also pronounced at site 4 on the Glen in July, and were linked to significant increases in the population density of the competitive amphipod, *G. pulex* (Figure 5.15(i)). Whilst the strength of biotic interactions was not measured in the current investigation, the idea that increasing population densities will increase biotic pressures is a fundamental ecological concept, with Darwin considering a 'struggle for existence' as inevitable following a population increase (Darwin, 1859, p. 41), and Solomon (1949, p. 13) stating that 'cannibalism and all forms of intraspecific competition, tend to be intensified as density increases'. This principle remains widely accepted and is considered as applicable to freshwater ecosystems, with experimental work suggesting that many benthic invertebrates have a preference for low population density substrata (Peckarsky, 1979; Holomuzki *et al.*, 2010). In particular, cannibalism has been demonstrated as density dependent in fish (Meffe, 1984; Nilsson, 2001) dragonfly nymphs (van Buskirk, 1989) and estuarine crustaceans (Moksnes *et al.*, 1997). Whilst explicit data is lacking for freshwater amphipods, intraguild predation between gammarids has been shown to be density dependent (Savage, 1996). It is therefore considered highly likely that the increasingly high *G. pulex* population densities represented an increase in the strength of biotic interactions including competition, predation and cannibalism in the benthic sediments as flow recession progressed, with adverse biotic conditions acting as a potential trigger of vertical migrations into the hyporheic zone. Refugium use was therefore expected at sites 2-5 on the Lathkill (Table 7.2), whilst low refugial capacity sediments and low disturbance magnitude were predicted to limit refugium use at Lathkill site 1 and Glen site 4 respectively (Table 7.1).

7.7.2 Evidence of refugium use

On the Lathkill, a month after *G. pulex* population densities started to rise in the benthic sediments, the taxon also started to become significantly more abundant in the hyporheic zone (Figure 5.20(i)), providing evidence of either passive range extension or true refugium use (i.e. active shelter seeking behaviour; Figure 7.4).

Here, the latter is argued as the most likely explanation, for several reasons. Firstly, when anomalous site 1 (see section 7.6.2) was excluded from analysis of the Lathkill, the proportion of the total *G. pulex* population inhabiting the hyporheic zone increased significantly between May and August, demonstrating that the rate at which individuals were migrating into deeper sediments had increased (Wood *et al*., 2010; Figure 7.4). Secondly, experiments have demonstrated that gammarids exhibit a behavioural response following exposure to chemical cues released by both potential predators (Åbjörnsson *et al*., 2000) and conspecific victims of cannibalistic attack (Wisenden *et al*., 2001), and such behavioural responses have been shown to include changes in habitat selection, in particular, migration into habitat with smaller interstitial spaces (McGrath *et al*., 2007). Thirdly, at sites 2-5, the shallow hyporheic sediments of the Lathkill were identified as having a high refugial potential, indicating that they provided a favourable habitat in which to shelter (Table 7.1).

Juvenile gammarids may be most likely to migrate into the hyporheic zone, as they are at particular risk of intraspecific predation (Dick, 1995; MacNeil *et al*. 1999), are smaller and so morphologically better suited to inhabitation of interstitial spaces, and have been demonstrated experimentally to use these spaces for protection from cannibalism. However, the hyporheic sampling method used in the current study has an inherent bias towards collection of smaller individuals (Fraser and Williams, 1997; Scarsbrook and Halliday, 2002) and it was therefore not appropriate to assess variation in refugium use by different age/size classes.

On the Glen, a concurrent increase in the benthic and hyporheic abundance of *G. pulex* followed habitat contraction at site 4 (Table 6.11; Table 6.16). However, these changes were localised, abundances remained considerably lower than those reported on the Lathkill, temporal change was not significant, and the hyporheic proportion of the population did not change; evidence of a consistent pattern of refugium use between rivers is therefore limited.

7.8 Use of the hyporheic zone refugium during spates

The hyporheic zone was first formally proposed as a refugium during high flows following the finding that invertebrates occurred deeper in the sediments after a spate, thus reducing their risk of displacement (Williams and Hynes, 1974) and similar observations had also been made the previous decade (Clifford, 1966). Since this early work, several other studies have contributed additional evidence for the ‘flood refuge hypothesis’ (Boulton *et al*., 2004), including Dole-Olivier and Marmonier (1992a), Holomuzki and Biggs (2000) and Bruno *et al*. (2009). However, increases in the hyporheic abundance of benthic invertebrates are not always observed during high flow events (Imbert and Perry, 1992; Gayraud *et al*., 2000), or may be restricted to certain taxa (Marchant, 1995; Lancaster, 2000) or to hyporheic sediments that meet certain environmental criteria (Dole-Olivier *et al*., 1997). In addition, high magnitude spates can reduce the abundance of permanent hyporheic residents (Olsen and Townsend, 2005; Hancock *et al*., 2006) as well as hampering downward migration of benthic taxa.

7.8.1 Potential triggers of refugium use

The Lathkill spate was identified as having a substantial impact on the benthic invertebrate community (Figure 5.14), whilst the high-flow events on the Glen had detectable impacts on larvae of the families Chironomidae and Simuliidae (Table 6.11). In the Lathkill, the capacity of the hyporheic zone to function as a refugium had been demonstrated during the flow recession, indicating that the physical environment of these sediments was able to support refugees, and inhabitation of the hyporheic zone had the potential to reduce the impacts of spates in both rivers. However, rapid disturbance onset on both rivers and low disturbance magnitude on the Glen resulted in no refugium use being expected (Table 7.2), even in sediments with high refugial capacity (Table 7.1).

7.8.2 Evidence of refugium use

In the Lathkill, there was no significant increase in the hyporheic abundance of any predominantly benthic taxon after the spate, with most taxa instead experiencing hyporheic population reductions, and for *G. pulex* this decline was of a magnitude approaching that observed in the benthic sediments (Table 5.18). The only taxon to show any overall increase in hyporheic abundance between August and September was the ubiquitous Oligochaeta (Table 5.18). In contrast, localised increases in the abundance of some common hyporheic taxa in benthic sediments in September, as well as a moderate reduction in fine sediment concentrations, suggested an increase in the strength of upwelling water during the spate. Disturbance magnitude (including peak velocity, shear stress and bedload movement) as well as behavioural responses to an environmental stress have been shown to vary over small spatial scales (Palmer *et al*., 1996; Lake, 2000; Oldmeadow *et al*., 2010). It is therefore probable that both features of the habitat itself (e.g. fine sediments, upwelling water) and disturbance-related parameters (e.g. sediment mobilisation, rate of flow increase, peak velocities), differed between sampling points to result in the observed lack of active refugium use.

In the Glen, two taxa declined in abundance in the benthic sediments following the spate, and one, Simuliidae larvae experienced concurrent significant increases in both hyporheic abundance and the hyporheic proportion of the total community, indicating active refugium use (Tables 6.19; Figure 7.4). Simuliidae are filter feeders, typically attaching to submerged macrophytes or boulders, and as such their occurrence in the hyporheic zone initially appears anomalous (Poole and Stewart, 1976; Giberson and Hall, 1988). However, as individuals that align themselves with the flow to feed, simuliids are at particular risk of displacement during spates (Maitland and Penney, 1967), and therefore need behavioural adaptations such as refugium use to facilitate persistence in a habitat. Simuliidae larvae can travel from exposed feeding position to the sediment surface by means of silk anchor threads and then use ‘looping’ movements to enter interstitial spaces (Wotton, 1979). Despite filtering being the family’s dominant mode of feeding, simuliids also consume deposited organic matter (Courtney, 1986; Giberson and Hall, 1988), which is plentiful in the hyporheic zone. Indeed, early instars hatching in interstitial spaces do not possess head fans and are obligatory deposit feeders (Giberson and Hall, 1988), numerous studies report their occurrence in the hyporheic zone where other resources are limited (e.g. Jeffrey *et al*., 1986; Malard *et al*., 2003), and certain specialist taxa are obligate hyporheos with adaptations to a subterranean existence (Courtney, 1986). The hyporheic zone, whilst not necessarily the taxon's preferred habitat, therefore appears capable of supporting Simuliidae, and previous studies have also reported its use as a spate refugium (Richardson and Mackay, 1991). In the Glen, restriction of refugium use to the Simuliidae may reflect the taxon's particular risk of displacement.

7.9 Use of the hyporheic zone refugium following streambed drying

Due to its potential retention of free water, streambed drying is one of the principle conditions during which the hyporheic zone is proposed to act as a refugium. However, whilst several previous studies have noted evidence of active use of the hyporheic zone to aid survival during such dry phases (Boulton *et al*., 1992; Cooling and Boulton, 1993; Griffith and Perry, 1993; Clinton *et al*., 1996), other studies have noted no such refugium use (Smock *et al*., 1994; Del Rosario and Resh, 2000), this commonly being attributed to inappropriate environmental conditions in the hyporheic zone (Smock *et al*., 1994; Boulton and Stanley, 1995; Belaidi *et al*., 2004).

7.9.1 Potential triggers of refugium use

Complete streambed drying occurred at site 4 on the Glen between the July and August sampling dates and again prior to September sampling (Figure 6.2), whilst loss of surface water affected two marginal sampling areas on the Lathkill in August (section 5.4.1). On the Glen, complete loss of surface water was a high magnitude disturbance that was expected to result in refugium use despite low-refugial potential sediments; in contrast, drying on the Lathkill was localised and laterally connections with submerged surface habitats were retained, so refugium use was not considered likely (Table 7.2).

7.9.2 Evidence of refugium use

Sampling was undertaken *during* marginal drying on the Lathkill, but not until several days *after* surface flow had resumed on the Glen; vertical distribution of invertebrates in the Glen may therefore have changed in the period between the dry phase and sampling. Active refugium use, as evidenced by concurrent increases in hyporheic abundance and hyporheic proportion (Wood *et al*., 2010; Figure 7.4) was not observed for any taxon on either river, with the exception of minor increases in both metrics for the Oligochaeta in the Glen (Table 6.16; Table 6.18). Declines in benthic abundance of all other common taxa were accompanied by comparable reductions in hyporheic abundance in this river. However, since sampling was undertaken after the event, the benthic community, whilst depauperate, may have included individuals that had persisted during the dry phase in the hyporheic zone and subsequently migrated back to the surface. On the Lathkill, hyporheic community composition appeared to remain similar before and after drying of marginal areas (Figure 5.16), but insufficient data is available to infer general patterns of change in vertical distribution. However, particularly high taxon richness was recorded in two samples pumped from below dry sampling areas (17 and 14 taxa compared with an overall mean of 6.6 taxa 6 L^{-1}). The presence of a diverse range of taxa within the hyporheic zone both during and following drying disturbances indicates that the hyporheic zone is passively used as a refugium even when active migrations do not occur (see section 7.11; Clifford, 1966; Imhof and Harrison, 1981; Fenoglio *et al*., 2006).

7.10 Expected vs. observed spatial variability in the refugium use

The preceding discussion detailed general patterns of refugium use during spate, streambed drying and low flow disturbances. However, spatial variability in refugial potential meant that refugium use was expected to differ between sites during a particular disturbance (Table 7.2).

During spate events, no active use of the hyporheic zone refugium was expected, due to rapid disturbance onset on the Lathkill nullifying even high refugial capacity, whilst low disturbance magnitude was considered insufficient to warrant use of low-quality refugia on the Glen (Table 7.2). Observations were largely in accordance with this expectation, with no refugium use observed despite substantial benthic community losses on the Lathkill, and only taxon-specific effects on the Glen benthos; this suggested that explanations of the expected lack of refugium use were essentially valid. However, not only did the hyporheic abundance of benthic invertebrates not increase after the Lathkill spate, abundance actually declined, indicating the displacement of flow recession refugees including *G. pulex* (Table 5.18). The argument that benthic invertebrates did not have time to respond to the rapid rise in discharge can be only be used to explain the lack of any further increase in their hyporheic abundance; in the Lathkill, it does not explain the failure of the hyporheic zone to protect those invertebrates already residing within it. This may be explained by disturbance related changes in environmental parameters, in particular bedload movement of hyporheic sediments and localised increases in the strength of upwelling water.

Other disturbances also resulted in the expected refugium use being observed (Table 7.2). Following ponding at Glen site 3, no refugium use was expected and none was observed, presumably due to the adverse conditions in the surface channel (low oxygen availability, low flow velocities) being more pronounced in the hyporheic zone. Other disturbing forces were related to flow recession and consequent habitat contraction, which resulted in increases in benthic invertebrate population densities at all sites on the Lathkill and at site 4 on the Glen, with a resultant increase in biotic interactions being highly likely in the Lathkill. Despite the comparable disturbing forces, refugium use was not expected at all sites. In particular, refugium use was expected and observed at Lathkill sites 2 to 5, where high refugial capacity hyporheic sediments provided a suitable alternative to the moderate magnitude biotic disturbance in the surface stream; this same disturbance, however, was insufficient to trigger migration into low refugial potential sediments at site 1. This observed spatial variability in the refugial capacity of the hyporheic zone during low flows is detailed in Figure 7.3.

Two predictions of refugium use were not supported by the data (Table 7.2). Firstly, use of the hyporheic zone was expected at Glen site 4 following streambed drying; this was a high magnitude, slow onset disturbance which was considered sufficiently severe to trigger vertical migrations into the hyporheic zone despite low refugial capacity (Table 7.2). However, the expected active migrations were not observed, with a decline in hyporheic abundance instead being observed for all common taxa except the Oligochaeta. This lack of observed refugium use followed an increase in the hyporheic abundance of many taxa between June and July, which had demonstrated the capacity of these sediments to support higher abundances of benthic invertebrates. This change in refugium use suggests that some abiotic condition developed in the hyporheic sediments as a result of the drying disturbance which further reduced refugial potential. Some previous studies have attributed a lack of refugium use during drying to a single variable of particular importance, such as the development of anoxia (Smock *et al*., 1994), the loss of free water (Boulton and Stanley, 1995), or the compaction of surface sediments (Belaidi *et al*., 2004). Hyporheic conditions were not characterised during the dry phase in the Glen, but the mean proportion of fine sediment at site 4 was high (36 %; Table 6.21) and compaction may have occurred; low oxygen availability was recorded after the resumption of surface flow and concentrations may have been even lower during the dry phase (Figure 6.7); and the responsiveness of this losing reach to changing hydrological conditions may have resulted in loss of free water from the shallow hyporheic sediments. Any one of these single factors would be sufficient explanation for the lack of refugium use. The lack of faunal migrations into the hyporheic zone in this instance highlights the ability of disturbance-related variability in habitat parameters to override usual refugial potential.

The second incorrect prediction related to use of the hyporheic refugium following the Glen spates: refugium use was not expected, but was observed in the Simuliidae (Table 6.18; Figure 6.19). This family is at particular risk of displacement during spates due to a preference for fast-flowing, exposed habitats, and the inaccuracy of this prediction highlights that the experience of a disturbance is taxon-specific (Holomuzki and Biggs, 2000), and generalisations of refugium use may often have exceptions. In many cases, however, it appears possible to predict refugium by comparing refugial potential (based on the physical characteristics of the hyporheic sediments; Table 7.1) with disturbance characteristics (principally magnitude and rate of change; Table 7.2).

7.11 Defining the behaviours controlling refugium use

Previous studies have differed in their definition of what constitutes refugium use. Some studies have reported an increase in numerical *abundance* of a predominantly benthic taxon as evidence of *active* migration into deeper sediments (e.g. Williams and Hynes, 1974; Marchant, 1995; Clinton *et al*., 1996); however, it can be argued that whilst this may be true, in some cases it indicates only population expansion (Figure 7.4). Others have considered an increase in the hyporheic *proportion* of a taxon’s total (benthic + hyporheic) population as evidence that the hyporheic zone promotes invertebrate survival (Griffith and Perry, 1993; Fenoglio *et al*., 2006; Wood *et al*., 2010). Whilst it is true that survival may be enhanced for those invertebrates already inhabiting the hyporheic zone at the onset of a disturbance, this refugium use may be *passive* since an increase in proportion can include a reduction in abundance compared with pre-disturbance conditions, so long as the decline is of a lesser magnitude than occurs in the benthic sediments. Therefore, regardless of benthic population dynamics (increase, decrease or no change in abundance), the most compelling evidence of refugium use (i.e. active shelter-seeking behaviour) is provided by concurrent increases in hyporheic abundance and hyporheic proportion (Figure 7.4; Wood *et al*., 2010).

In the current study, evidence of active refugium use was observed at sites 2-5 on the Lathkill during the flow recession, with significant increases being recorded in *G. pulex* benthic abundance, hyporheic abundance, and the hyporheic proportion of the community (Figure 7.4). Active refugium use was also recorded in the Simuliidae on the West Glen following the spate, and in this case, increases in hyporheic abundance and proportion were accompanied by a reduction in benthic abundance. In contrast, whilst benthic and hyporheic abundance both increased at Glen site 4 following habitat contraction, the hyporheic proportion of the population did not change, and so the increase in hyporheic abundance is considered as passive range

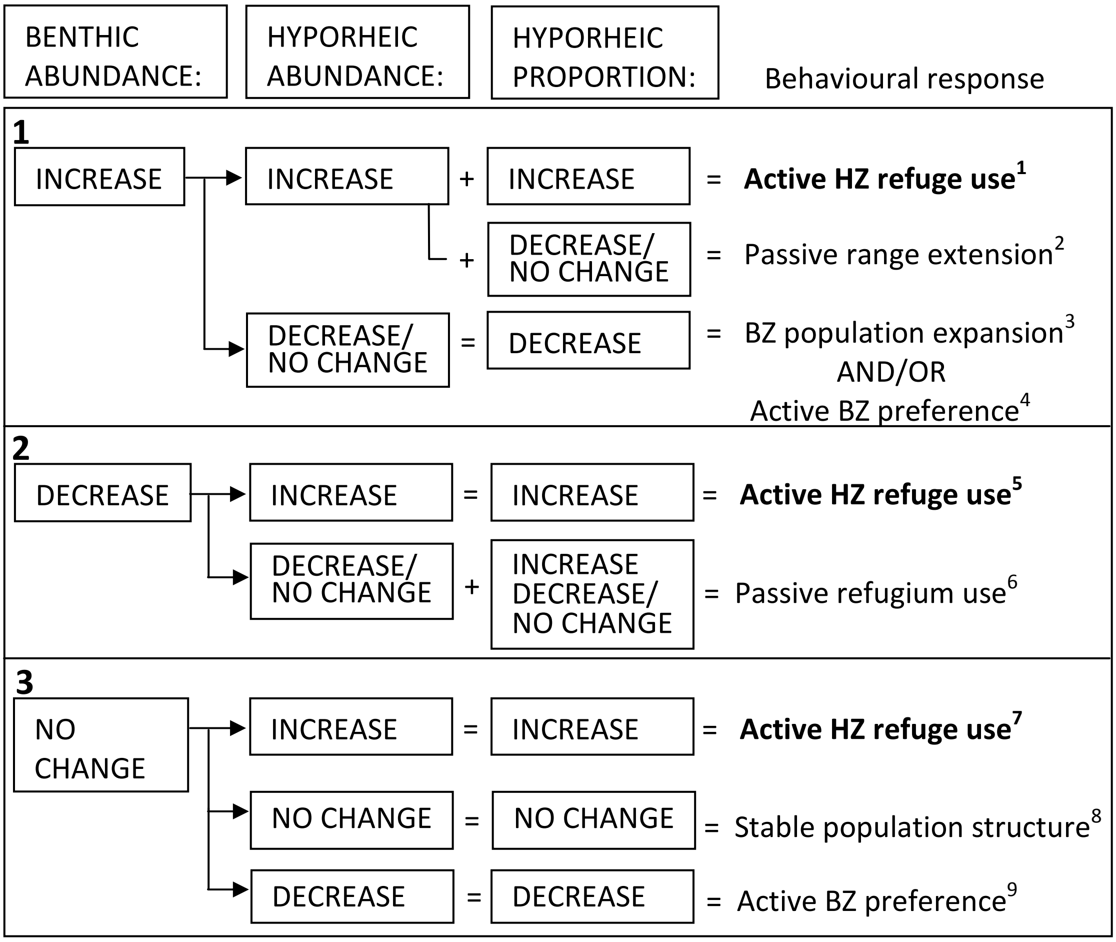


Figure 7.4: Behavioural responses inferred from changes in absolute and relative abundance of benthic and hyporheic invertebrates. HZ = hyporheic zone; BZ = benthic zone. Examples: [1] Wood *et al*., 2010, **Lathkill sites 2-5 during flow recession**; [2] **Glen site 4 during habitat contraction**; [3] **Lathkill site 1 during flow recession**; [4] Dole-Olivier and Marmonier, 1992a; **[5] Glen sites 1-2 following spates (Simuliidae)**, Marchant, 1988, Clinton *et al*., 1996; Stubbington *et al*., 2009a; [6] Griffith and Perry, 1993, Fenoglio *et al*., 2006, James and Suren, 2009; **Lathkill after spate**; **Glen site 4 after streambed drying**; [7]No known examples; [8] James *et al*., 2008, **Glen all sites during spates**; [9] No known examples.

extension (Figure 7.4). Another contrasting pattern was observed at Lathkill site 1, where flow recession was associated with a substantial increase in the benthic abundance of *G. pulex*, but this was not accompanied by any increase in the taxon's hyporheic abundance, and the hyporheic proportion of the population therefore declined, indicating an active preference for benthic over hyporheic sediments. Following both the high magnitude Lathkill spate and streambed drying on the Glen, invertebrate densities declined in both benthic and hyporheic sediments; however, the hyporheic zone may nonetheless have promoted survival of invertebrate fauna through their passive refugium use, i.e. protection of those present by chance during adverse conditions in the surface stream (Figure 7.4).

This study therefore provides evidence that in addition to protecting those invertebrates that active seek refuge there, the hyporheic zone also promotes invertebrate survival through passive refugium use (comprising both temporally stable occupation of the hyporheic zone and passive migrations initiated by high-flow disturbances). Both active and passive refugium use may enhance benthic invertebrate survival during an adverse condition in the surface stream (Holomuzki and Biggs, 2000), and the importance of these modes of refugium use depends on the nature of the disturbing forces. The nature of a behavioural response can be determined from benthic abundance, hyporheic abundance and hyporheic proportion using the model outlined in Figure 7.4.

7.12 Summary

A range of conditions were identified as potential invertebrate stressors on the River Lathkill and River Glen, including spates, streambed drying and habitat contraction-related increases in biotic interactions. In all cases, exposure of benthic invertebrates to the disturbing forces could have been reduced by migration into the hyporheic zone. However, spatial variability in the environmental characteristics of the hyporheic zone (hydrologic exchange, oxygen availability, proportion of fine sediments) resulted in refugial potential varying between sites, and characteristics of the disturbing forces (magnitude, rate of onset) also influenced refugium use. As a result, use of the hyporheic zone was found to be restricted to certain taxa and certain conditions, particularly *Gammarus pulex* during the increase in biotic interactions and Simuliidae during spate events. In many cases, a combination of environmental characteristics and disturbance-related parameters was successfully used to predict the occurrence of refugium use. However, incorrect predictions highlighted the shortcomings of making generalisations, firstly regarding the response of communities including numerous taxa, and secondly regarding the effects of inherently unpredictable disturbing forces.

8. The hyporheic zone as an invertebrate refugium: wider context and future directions

8.1 Introduction

In this chapter, key findings from the thesis are considered in the context of the wider research area. The contribution made to understanding of the ecological functioning of the hyporheic zone is described and the consequent need to protect this habitat is emphasized. Whilst all aims of the study were met (section 1.2; also see section 7.1), invertebrate responses to identified stressors were taxon-specific and depended on fulfilment of environmental and disturbance-related criteria (sections 7.6-7.9). These inconsistent results highlight the limitations of single ecosystem components (such as the hyporheic zone) in promoting invertebrate survival, and the importance of maintaining a heterogeneous range of instream refugia is discussed. The strengths and limitations of the current investigation are then used to suggest priorities for future research projects. The paired benthic-hyporheic approach used in the current study is recommended to facilitate interpretation of invertebrate responses to environmental variability, in conjunction with the behavioural response interpretation tool proposed in Figure 7.4. This approach should be simultaneously applied to multiple potential refugia, to aid understanding of refugium use at community and ecosystem levels. However, the natural trajectory experiment used in the current study (section 4.2) could not isolate specific factors determining refugium use; an experimental approach at the micro-scale (individual organisms and their immediate surroundings) should be pursued to improve understanding of the factors influencing invertebrate behaviour.

8.2 The hyporheic zone as an invertebrate refugium

In this section, the contribution made by the thesis research to understanding of the ecological functioning of the hyporheic zone is outlined, and the consequent importance of maintaining the ecological integrity of the zone is emphasized.

8.2.1 A new role for the hyporheic refugium

Previously, the ecological role of the hyporheic zone as a refugium for benthic invertebrates has been proposed (Orghidan, 1959, 2010), formalised in the Hyporheic Refuge Hypothesis (Williams and Hynes, 1974), demonstrated during adverse hydrological conditions including spates (Clifford, 1966; Dole-Olivier *et al*., 1997) and streambed drying (Boulton *et al*., 1992; Fenoglio *et al*., 2006), and remains accepted as a central ecological function (Robertson and Wood, 2010). Small interstitial spaces, including those in the hyporheic zone, have also been shown to protect vulnerable invertebrates (e.g. early instars and small individuals) from biotic pressures such as predation and cannibalism (Jacobi and Cary, 1996; McGrath *et al*., 2007). The results from the River Lathkill are the first to link these two previously understood roles of the hyporheic zone: refugium use related to hydrological conditions and refugium use related to biotic pressures (following flow recession and habitat contraction). In making this link, some of the first evidence of the hyporheic zone as a refugium during moderate hydrological conditions is described. This research therefore provides new evidence of the hyporheic zone as a vital contributor to total ecosystem functioning, adding further weight to the argument that these subsurface sediments should be explicitly considered by freshwater monitoring programmes and holistic restoration schemes aiming to maximise habitat heterogeneity (Boulton, 2007a; Kasahara *et al*., 2009).

8.2.2 The increasing importance of the hyporheic refugium

The hyporheic zone's role as a refugium during low flow conditions is of particular relevance in the face of future climate change scenarios. In the past century, UK air temperatures have risen by 0.8 ^{o}C, whilst precipitation has become increasing seasonal, with greater winter and reduced summer rainfall (Marsh *et al*., 2007; Jenkins *et al*., 2009). Whilst evidence of consequent changes in streamflow remains equivocal at the low flow end of the hydrological continuum, there is some suggestion of a decrease in the magnitude of low flow conditions (Hisdal *et al*., 2001; Hannaford and Marsh, 2006). Future scenarios predict continued increases in temperature and decreases in summer rainfall in the regions studied (Shackley *et al*., 2001; Blenkinsop and Fowler, 2007), and whilst future changes in river flows remain uncertain (Chun *et al*., 2009), it seems probable that many rivers will experience reduced summer discharge (Arnell, 2003; Fowler and Kilsby, 2007). Anthropogenic pressures on surface and groundwater resources are also predicted to increase, exacerbating the severity of reductions in discharge (e.g. Fowler *et al*., 2007).

In response to predicted climatic change, the hyporheic zone may become an increasingly important refugium following streambed drying in intermittent streams, including previously perennial streams that experience shifts to intermittent flow (Stanley and Valett, 1991; Wood and Petts, 1999). In systems retaining perennial flow, lower flows may mean reductions in habitat availability and concentration of invertebrate fauna, such as observed in the Lathkill and Glen. Covich *et al*. (2003) have explicitly stated that instream biotic interactions are likely to increase at the low flow end of the hydrological continuum due to climatic variability and anthropogenic demand for water resources. In such cases, the hyporheic zone may play an increasingly important role in protecting vulnerable individuals from biotic pressures.

8.2.3 Maintaining the ecological integrity of the hyporheic zone

With adverse conditions in the surface channel set to increase in magnitude and frequency, there is an ecological impetus to maintain effective functioning of the hyporheic zone and maximise its capacity to function as a refugium. In addition, to combat the ecological consequences of increasing anthropogenic and climatic pressures, new legislative drivers have been introduced that protect lotic ecosystems. Of particular relevance is the EU Water Framework Directive (CEC, 2000), which requires a more holistic approach to river ecosystems management than has previously been prescribed (Wharton and Gilvear, 2006). As a vital contributor to stream ecosystem functioning, there are now legal incentives to maintain effective functioning of the hyporheic sediments (CEC, 2000). In practice, this means ensuring that hydrologic exchange between the surface stream and the groundwater aquifer can occur unimpeded through the hyporheic zone, which in turn requires prevention or restoration of sediments clogged with fine material (Boulton, 2007a). It is the interconnectivity of the benthic and hyporheic sediments that allows invertebrates to migrate into the hyporheic zone, and only interstices which freely exchange water, nutrients, food resources and organisms with adjacent ecosystem components provides a suitable habitat for benthic fauna (Vervier *et al*., 1992; Brunke and Gosner, 1997).

To date, most projects aiming to restore the ecological integrity of river ecosystems have focussed on the surface stream and the benthic fauna (Bannister *et al*., 2005, Boulton, 2007a). Whilst such efforts may well have positive impacts on subsurface sediments (Boulton *et al*., 2010), it is now recognised that efforts targeting the restoration or maintenance of hyporheic exchange flows are also required (Jansson *et al*., 2007; Boulton, 2007a).

8.3 The hyporheic component of instream refugia

In the current investigation, use of the hyporheic zone refugium was limited during both the Lathkill spate and streambed drying on the Glen; in both cases, this was linked in part to features of the disturbing forces (Table 7.2). However, the hyporheic zone is only one of a range of refugia potentially able to promote invertebrate survival during instream disturbances, and limited use of the subsurface sediments in the current study may also be partly explained by use of alternative refugial habitats. The range of refugia available differs depending on the nature of the disturbing forces, but in all cases, habitat heterogeneity (i.e. patchiness) is recognised as lowering the detrimental effects of physical disturbance on instream communities (Sousa, 1984; Lancaster and Belyea, 1997; Negishi *et al*., 2002).

8.3.1 Alternative spate refugia

During spates, areas within and features of the surface channel can also act as refugia from high flows (Figure 8.2). Flow refugia include dead zones (Lancaster and Hildrew, 1993b, 1994; Lancaster, 1999; Rempel *et al*., 1999) and inundated floodplain areas (Townsend *et al*., 1997; Matthaei and Townsend, 2000). Instream features that can protect against displacement include large, stable substratum particles (Townsend, 1989; Cobb *et al*., 1992; Matthaei *et al*., 2000), microform bed clusters (organised groups of surface stones that are resistant to entrainment; Reid *et al*., 1992; Matthaei and Huber, 2002), woody debris (Palmer *et al*., 1996) and riparian vegetation (Robinson *et al*., 2004). In the current investigation, velocity measurements were largely taken from run and riffle habitats, and so slow flowing areas within the surface channel were not formally identified. Entrance into a laterally or longitudinally (as opposed to a vertically) located refugium may be achieved through entrance into drift, which during spates is likely to include both catastrophic (i.e. involuntary) and behavioural (i.e. voluntary) components (Brittain and Eikeland, 1988). Whilst drift may be an effective means of entering slow-flowing refugia in rivers that maintain a connection with the floodplain, in anthropogenically-altered rivers such as the Lathkill and Glen, there is a reduced likelihood of such a strategy promoting survival, and a lack of flow refugia has been associated with more pronounced impacts of spate events in channelized compared with natural reaches (Negishi *et al*., 2002).

Various factors may have increased the likelihood of entrance into the drift over vertical migration during the Lathkill spate. Firstly, following an increase in benthic habitat availability during the moderate discharge increase that preceded the large spate (Figure 5.2), flow recession refugees may have migrated back to the surface channel and been resident in the surface sediments at the onset of the spate. Regardless of precise location in the sediment profile, changing flow velocities are recognised as initiating a drift response (Minshall and Winger, 1968; Ciborowski *et al*., 1977), and between the August and September sampling dates, discharge (and therefore, presumably in a constrained channel, also velocity) increased, decreased, then increased very sharply. Drift densities have also been shown to be greater when turbidity is higher (Ciborowski *et al*., 1977), when sediments are mobilised (Holomuzki and Biggs, 2000) and when a spate follows a prolonged period of flow stability (Irvine, 1985; Perry and Perry, 1986). These factors (rapid rate of velocity increase; multiple changes in velocity; high turbidity; antecedent conditions) may all have increased the likelihood of invertebrates entering into drift as opposed to migrating deeper into the hyporheic sediments. However, both the Lathkill and the Glen are artificially constrained within steep banks over much of their lengths, severing links with the floodplain, limiting the range of alternative refugia available, and reducing the probability of drifting invertebrates entering a refugial area (Power *et al*., 1988; Sedell *et al*., 1990).

8.3.2 Alternative drying refugia

Following streambed drying, the range of potential refugia that retain free water or high levels of moisture include crayfish burrows, woody debris, algal mats, large substratum particles and receding pools (Boulton, 1989; Stubbington *et al*., 2009b). In the Glen, woody debris was present at the sampling site whilst other potential refugia were not observed but may have been available up- or downstream. Both increases and decreases in flow velocity can initiate invertebrate entrance into drift (Minshall and Winger, 1968; Corrarino and Brusven, 1983; Perry and Perry, 1986), and longitudinal rather than vertical migrations may have promoted survival during streambed drying on the Glen (Delucchi, 1989; Stanley *et al*., 1994), although invertebrates would have needed to drift for several kilometres to reach perennially flowing habitat (Maddock *et al*., 1995; Figure 3.6). Similarly, following localised drying on the Lathkill, the lack of evidence for active refugium use suggests lateral or longitudinal migrations into submerged surface habitats may have been used to promote survival instead of vertical migrations.

8.3.3 Alternative responses to increased biotic pressures

Following an increase in biotic pressures, vertical migration is only one survival strategy that invertebrates can use to enhance survival. In particular, other studies cite behavioural drift as a common (intra- and interspecific) predation avoidance response (Malmqvist and Sjöström, 1987; Brittain and Eikeland, 1988), and previous research has inferred density-dependent downstream migrations of *G. pulex* as a mechanism to reduce intraspecific biotic pressures at high population densities (Macan and Mackereth, 1957). However, drift generally declines in importance with a reduction in discharge, as demonstrated with specific reference to *G. pulex* by Elliott (2002; but see Williams and Moore, 1982) and low flows may not have promoted drift on the Lathkill. Gammarids are also known to actively migrate upstream (Hynes, 1960; Minkley, 1964; Williams and Williams, 1993) although cues triggering such behaviour remain unclear.

8.3.4 A conceptual model of hydrologically-mediated refugium use

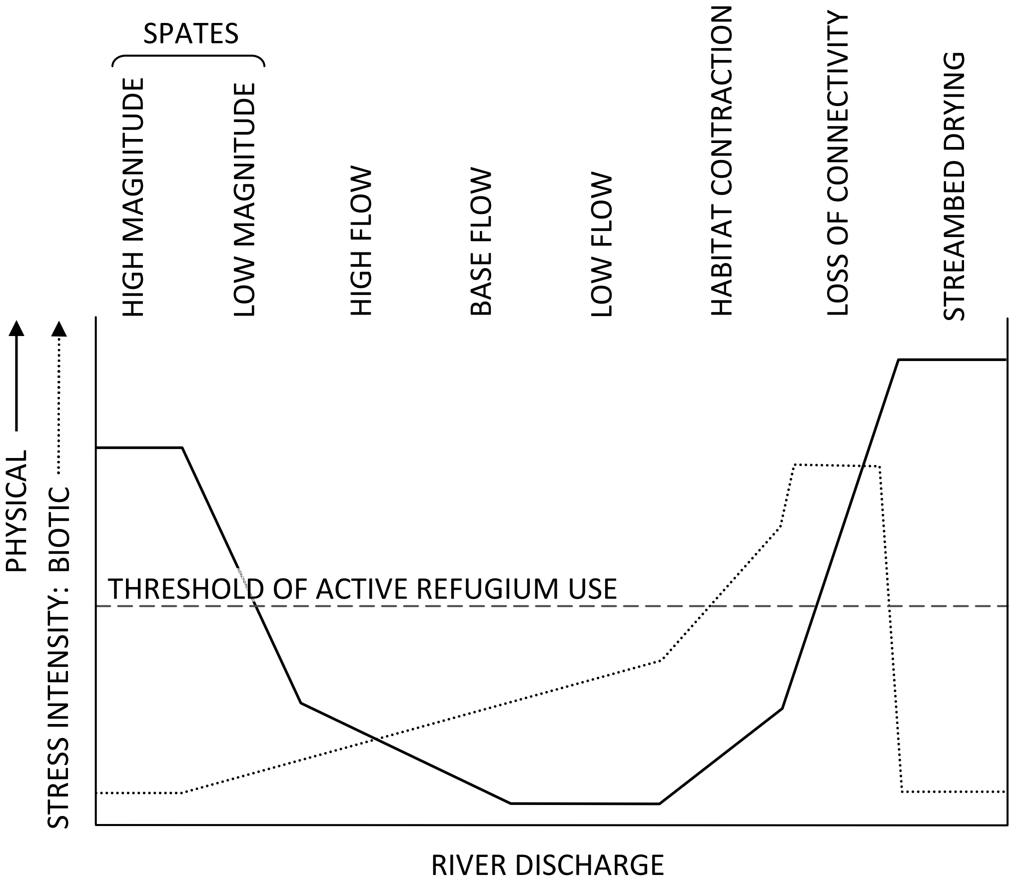


Figure 8.1: Conceptual model of changes in the physical and biotic stress intensity experienced by benthic invertebrates as river discharge declines (from left to right).

The intensity of both physical and biotic stress changes in response to variation in river discharge, potentially culminating in active migrations into various refugial habitats (Figure 8.1). At one extreme of the hydrological continuum, high magnitude spates represent periods of high intensity physical forces in the benthic sediments, precluding biotic interactions as determinants of community composition. As spate magnitude falls, impacts on invertebrate assemblages decline but physical factors remain the key influence on community structure. As discharge continues to decline from high flow to low flow, hydrological conditions become increasingly favourable for many competitive taxa and the key influence on community composition becomes the intensity of biotic interactions. Overall community stress is relatively low during these moderate flows; however, if stable base flow or low flows continue for a prolonged period, biotic stress may become increasingly elevated. In addition, if flow recession results in submerged habitat contraction, biotic interactions increase sharply as invertebrates become concentrated. As loss of connected surface flow restricts biota to isolated pools, exposure to biotic stress peaks whilst physical stress (e.g. low dissolved oxygen availability) continues rising. If contraction of isolated pools culminates in complete streambed drying, particularly harsh physical conditions become the dominant influence on community composition, with a consequent reduction in biotic interactions.

Towards both the high and low flow extremities of the hydrological continuum, physical and/or biotic stress intensity increases past a threshold which triggers shelter-seeking behaviour; this threshold may vary between taxa, age/size classes and genders (Figure 8.1). Movement into various lower-stress refugia may occur, although the ability to enter these specific habitats is dependent on both environmental and disturbance-related criteria being met.

8.3.5 Importance of maintaining a full range of refugia

The hyporheic zone is therefore only one ecosystem element that can promote invertebrate survival during adverse conditions in the surface stream (Figure 8.2), and its refugial capacity is dependent on environmental and disturbance-related criteria being met (Table 7.1; Table 7.2). In addition, whilst the current investigation found evidence of *passive* refugium use in a variety of taxa during all adverse surface conditions, evidence of *active* refugium was limited to very few taxa (i.e. *Gammarus pulex* during habitat contraction on the Lathkill and Simuliidae larvae following the Glen spates; Figure 7.4). There is therefore a need to maintain total habitat heterogeneity including multiple refugia in stream ecosystems, and a range of the additional refugia should also be enhanced in holistic river rehabilitation schemes.

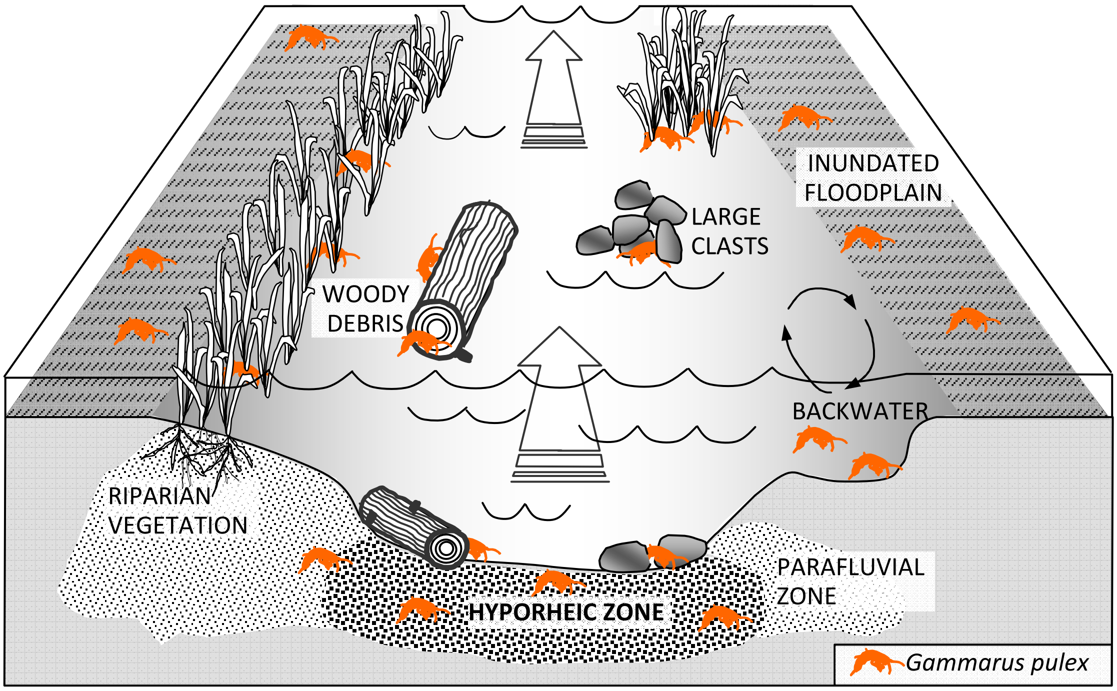


Figure 8.2: Three-dimensional conceptualisation of the hyporheic zone as one of several potential instream refugia available to invertebrates during high flows. Arrows indicate direction of flow. *Gammarus pulex* is used as a representative benthic invertebrate.

8.4 Landscape- to invertebrate-scale perceptions of the river ecosystem

Previous work has recognised the three spatial dimensions (longitudinal, lateral, vertical) of river ecosystems and thus the importance of a holistic perspective in explaining instream processes (Vannote *et al*., 1980; Junk *et al*., 1989; Ward, 1989; Stanford and Ward, 1993). A holistic approach is of particular relevance in investigations of hyporheic functioning, due to the connections these sediments make with adjacent ecosystem components (Stanford and Ward, 1993). Equally, linkages between naturally connected ecosystem components can be broken by anthropogenic activity, for example dam and weir construction can limit longitudinal connectivity (Ward and Stanford, 1983; Stanford and Ward, 2001), channelization can severe links with the surrounding floodplain (Sedell *et al*., 1990; Ward and Stanford, 1995) and sedimentation can clog hyporheic interstices and compromise vertical hyporheic exchange (Hancock, 2002; Kondolf *et al*., 2006). Severing of linkages in any dimension reduces the range of refugia available to instream invertebrates and so may exacerbate detrimental impacts of disturbance on stream fauna (e.g. Gagneur & Chaoui-Boudghane, 1991; Negishi *et al*., 2002); there is also the possibility that remaining refugia increase in importance during instream disturbances.

In response to a disturbance, benthic invertebrates can either: a) stay put; b) migrate longitudinally or laterally (upstream/lateral migration and/or downstream/lateral drift); or c) migrate vertically (into the hyporheic zone). The Lathkill (where evidence of active refugium use was widespread during flow recession) is highly modified, being constrained within reinforced banks and regularly interrupted by weirs, including a natural tufa barrier and several man-made structures (Figure 8.3). Such structures severely restrict, and often prevent, upstream migration of invertebrates including *G. pulex* (Kelly and Dick, 2005). Upstream of man-made weirs, ponded areas with silt-dominated substrata provide habitat for brown trout (*Salmo trutta*), an important invertebrate predator (MacNeil *et al*., 1999). Presence of predatory fish can also impede invertebrate movement, and has been shown to reduce drift in *G. pulex* (Andersson *et al*., 1986). Therefore, both longitudinal and lateral linkages have been cut in the Lathkill and the range of movement options available to invertebrate fauna reduced (Figure 8.3).

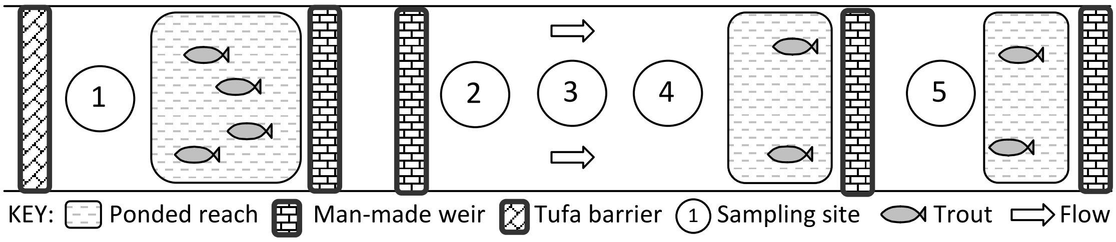


Figure 8.3: Plan-view conceptualisation of the River Lathkill study area, highlighting potential barriers to longitudinal invertebrate migration.

Despite the importance of a whole-stream perspective in understanding behavioural responses, an invertebrate's perception of its surroundings is always small-scale (Lancaster, 2008). The decision to stay put or migrate must therefore be made based on the immediate environment only, calling into question the idea that large-scale channel modifications can influence invertebrate behaviour. However, it is suggested that the inferred behaviour of *G. pulex* at Lathkill site 1, namely the failure of individuals to migrate into the hyporheic zone despite very high benthic population densities (Figure 7.4), may be partly explained by considering the wider riverscape. From site 1, moving >50 m downstream results in the migrant entering a silty, ponded area with high brown trout densities (pers. obs.), whilst those migrating roughly the same distance upstream encounter turbulent water at the base of the tufa barrier (Figure 8.3). It has been demonstrated that *Gammarus* will remain within a preferred substratum size class rather entering less suitable patches (Adams *et al*., 1987), and the particularly high benthic population densities at this site may therefore be explained by the lack of suitable habitat in either upstream or downstream directions (Figure 8.3).

8.5 Directions for future research

The ecology of the hyporheic zone is a young research field and understanding of its ecological attributes continues to grow year by year (Krause *et al*., 2009; Robertson and Wood, 2010). However, significant research gaps remain, and the realisation of both the ecological importance of the zone and the threats to its integrity render further research a high priority (Krause *et al*., in press).

8.5.1 Importance of an interdisciplinary approach

Considering the dependence of ecological integrity on effective hydrological exchange processes, future research into the ecological functioning of the hyporheic zone requires an interdisciplinary approach combining elements of ecology, hydrology, and sedimentology (Krause *et al*., in press). The current study, whilst ecological in focus, highlighted the importance of hydrological (upwelling vs. downwelling), sedimentological (coarse vs. fine sediments) and water chemistry (groundwater vs. surface water dominated) parameters in determining invertebrate use of the hyporheic zone (see Figure 7.3). It is recommended that future work build on the interdisciplinary approach taken both here and previously (e.g. Dole-Olivier *et al*., 1997), for example by combining biological sampling with a comprehensive mini-piezometer network capable of determining precise spatiotemporal variability in vertical hydrologic exchange (e.g. Käser *et al*., 2009); emerging technologies should also be exploited to map hydrologic exchange over multiple spatial scales (Boulton *et al*., 2010).

8.5.2 Benthic vs. hyporheic community responses

Ecological monitoring programmes, research projects, and restoration schemes typically have the underlying assumption that the response of the benthic community is representative of all instream invertebrates (Extence *et al*., 1987; Boon, 1988). However, this study and previous research (Wood *et al*., 2010) have demonstrated that benthic and hyporheic invertebrate assemblages may respond differently to the same environmental changes. Therefore, it is recommended that a paired benthic-hyporheic approach be applied across a wide range of research projects considering invertebrate community responses to changing environmental conditions. Firstly, this will ensure adequate characterisation of the hyporheic community, and secondly, will improve understanding of how community responses may differ. In particular, a paired benthic-hyporheic approach should be adopted to test the assumption that river rehabilitation projects have benefits for subsurface as well as surface fauna (Boulton *et al*., 2010).

8.5.3 Isolating the drivers of refugium use

The current project has also highlighted specific research gaps which require further attention. In particular, understanding of the capacity of the hyporheic zone to function as a refugium remains incomplete. At the ecosystem scale, use of the hyporheic zone is just one possible component of a community response to an instream disturbance, and future work should adopt a holistic approach by simultaneously characterising use of all potential refugia (Figure 8.2). In addition, whilst environmental parameters and disturbance-related factors have both been identified as determinants of refugium use, behavioural responses to other cues may be equally important; as Lancaster (2008) points out, the spatial patterns of invertebrate distribution reflect a multitude of individualistic events.

At the scale of the individual organism, it is not known what cues an invertebrate is responding to when it migrates into the hyporheic zone, actively enters the drift, migrates upstream or laterally, or remains in a location where an adverse condition develops (Death, 2008; Lancaster, 2008). In the Lathkill, *G. pulex* did not migrate into the hyporheic zone at groundwater dominated site 1 (Figure 5.20(i)), but it was not determined whether this was due to the direction of water movement, low oxygen availability, or another parameter. Field studies will need to be supplemented by experimental work to isolate single environmental factors and improve understanding of invertebrate responses to disturbance at the level of the individual and taxon. In both field and experimental studies investigating the drivers governing refugium use, a paired benthic-hyporheic sampling strategy is recommended, which will allow behaviour to be inferred from changes in benthic abundance, hyporheic abundance and hyporheic proportion using the tool outlined in Figure 7.4.

8.5.4 Long-term prospects for hyporheic refugees

In the current study, passive use of the hyporheic refugium was widespread, whilst evidence of active refugium use was inferred from changes in the hyporheic abundance and proportion of, firstly, the *G. pulex* population during the Lathkill flow recession and, secondly, the Simuliidae population following the Glen spates (Table 5.21; Table 6.18; Figure 7.4). However, on the Lathkill, many flow recession refugees were not protected from displacement during the subsequent spate (Figure 5.20(i)), whilst on the Glen, the benthic population of Simuliidae did not recover (possibly due to seasonal changes in abundance; Figure 6.19). Both of these results call the long-term efficacy of the hyporheic zone refugium into question. Whilst the ability of invertebrates to migrate through sediments in both vertical directions has been demonstrated (Bo *et al*., 2006), the ability of hyporheic refugees to migrate back to the surface has not been confirmed. Further investigation is required to determine the mid- to long-term survival prospects for refugees. As micro-scale technology becomes more financially viable, future research should aim to track individual organisms to ascertain more precisely how an individual's vertical positioning changes in response to environmental variability and biotic pressures (Whitfield-Gibbons and Andrews, 2004).

In the current investigation, hyporheic refugees in the Lathkill faced similar risk of displacement by high flows as those inhabiting the benthic sediments (Figure 5.15; Figure 5.20), whilst those on the Glen may have become desiccated if the hyporheic zone dried after the surface sediments. These results suggest that migration into the hyporheic zone may be a risky survival strategy. In particular, during streambed drying, invertebrates may need to migrate to increasing depths to remain submerged, but this may be prevented by increasingly impenetrable sediments (Poole and Stewart, 1976; McElravy and Resh, 1991); entrance into the drift in search of perennial waters may be an equally successful survival strategy. Calculating the probability of survival for invertebrates taking different pathways at the onset of a disturbance is another intriguing possibility, but remains unattainable until new technologies become available.

8.6 Recommendations for invertebrate sampling in the hyporheic zone

As recognition of the ecological importance of the hyporheic zone increases, it is imperative that consistent approaches are established for all sampling procedures, including macroinvertebrate collection. Limited guidance is provided in the recently published Hyporheic Handbook (Buss *et al*., 2009), and it is appropriate to expand on this published information in light of experience gained during the current study. It is intended that these recommendations inform sampling programmes developed by regulatory bodies, as hyporheic monitoring becomes more widespread in response to legislation such as the Water Framework Directive (CEC, 2000).

Although the pump sampling technique employed in the current project has limitations (see section 4.4.2), it is recommended for more widespread use. First, pump sampling equipment is simple to construct using low-cost components (i.e. manual bilge pump, wood board, hose pipe, plastic tubing). Second, this equipment is light and easily transported by one person, and can also be operated by a lone worker. One potential issue is sample contamination by surface fauna. In the current study, two operatives were present, one operating the pump and the second manually creating a seal between the sampling well and the inserted hose. However, lone working is common practice at some regulatory bodies and an alternative procedure is therefore required. It is suggested that tying a length of fabric at the sampling well-hose pipe junction may suffice. An alternative would be to sample beneath exposed sediments (Buss *et al*., 2009). However, reliance on specific micro-habitats may be restrictive, given the variety of lotic ecosystems in which sampling may be conducted.

Table 8.1: Summary recommendations for sampling of hyporheic macroinvertebrates

	Recommended	***Rationale***
Sampling technique	Pump sampling	Simple, cheap, fast, operable by lone worker
Sample volume	6 litres	Comparability with previous research
Sample depth	20 cm	Achievable in most habitats No benthic influence
Number of sampling points	4 per habitat type	Higher numbers show little improvement in community representivity

Two parameters requiring standardisation are sample volume and sampling depth. Whilst 6 litres is a somewhat arbitrary sample volume, it has been used successfully in the current investigation and previous studies (Boulton *et al*., 1992, 2004). It is therefore recommended that the 6 litre precedent established by Boulton *et al*. (1992) be continued. Regarding sampling depth, the current study found community composition to be similar at depths of 10, 20 and 30 cm. Sampling at a single depth of 20 cm is therefore recommended: 10 cm may increase contamination from the benthic zone in porous sediments, whilst 30 cm may render pipe insertion unnecessarily difficult. In addition, a decision needs to be taken regarding the number of samples required to characterise a site. The current project found no improvement in community characterisation above four samples per site. However, this number was selected to represent a single habitat type (i.e. a riffle or run) and a greater number may be required for complete site characterisation; operatives may need to make judgements based on site-specific characteristics and locations of benthic sampling points.

8.7 Key outcomes

The key achievements of the thesis research are summarised below:

- *Expansion of the hyporheic zone's role as a refugium.* The current study is the first to demonstrate that the hyporheic zone may act as an invertebrate refugium during low flows. This new observation of a previously hypothesized role was possible due to the extended nature of the sampling campaign. Refuge use was linked to a decrease in habitat availability and an associated increase in biotic interactions in the benthic sediments (Figure 7.3; Section 5.11.3; Section 7.7).
- *Demonstration of the dynamic nature of the hyporheic refugium*: Previous studies have sought to characterise the refugial capacity of the hyporheic zone on a simple yes/no basis. In contrast, the current study has considered benthic invertebrate use of the hyporheic zone over an unprecedented period which encompassed a range of hydrological conditions. This extended sampling campaign demonstrated the variable nature of hyporheic refugium use, with usage varying spatially (due to hyporheic conditions; Figure 7.3), temporally (due to disturbance-related parameters; Section 7.6.3), and between taxa.
- *Major test of the paired benthic-hyporheic sampling strategy*. Previous research has typically focussed on either benthic *or* hyporheic invertebrate communities, due to perceived difficulties in comparing assemblages sampled using different techniques. The research presented here constitutes the most extensive test of a paired approach to date, and has clearly demonstrated that interactions between populations in the two adjacent habitats can be analysed effectively by calculating the *hyporheic proportion* of a population (Section 5.7; Section 6.7).
- *Development of a tool for inference of invertebrate behaviour*. An additional advantage of the paired benthic-hyporheic sampling approach was demonstrated at the analysis stage. By comparing concurrent temporal changes in the abundance of the two populations, the nature of invertebrate inhabitation of the hyporheic zone (including both active and passive refugium use) could be determined (Figure 7.4; Section 7.11). Such behavioural analysis represents a theoretical advance in ecological hyporheic research.

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APPENDIX 1 Raw baseline survey data for the benthic macroinvertebrate community of the River Lathkill: i) sites 1-4; ii) sites 5-9. Site locations provided in Figure 3.7

i)

	Site 1			**Site 2**					**Site 3**					**Site 4**				
	1	**2**	**3**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Crenobia alpina	1				1	1		1	5	16	17			1		16		
Dendrocoelum lacteum														1				
Polycelis felina				33	3	4	12	13	5	23	20	3		4	3	43	14	17
Piscicola geometra																		
Ancylus fluviatilis				21	38	42	2	5	31	29	53	1		9		2		1
Lymnaea peregra	1	1	1				1	3	4		1							
Lymnaea truncatula																		
Sphaeriidae	32	3	3	1	10	6	8			3								
Zonitoides																		
Asellus aquaticus	6		2															
Asellus meridianus				3	3	3	3	1	3								1	
Gammarus pulex	808	928	1280	520	304	480	870	112	66	64	88	280	590	161	132	268	183	64
Baetis sp.			1	3	9	6		13		21	40	15	20	2	3	3	2	1
Serratella ignita				3	3			2	4	2	1		1		2			
Ecdyonurus sp.																		
Chloroperla torrentium																		
Leuctra fusca				26	4	12	1	2	5				2					
Leuctra hippopus														1				
Nemoura avicularis																		
Nemoura cambrica	1				1	8		4		2	1	2	3					
Nemurella picteti	1			1														
Protonemoura meyeri					1											2		
Nemouridae (early instar)																		
Dinocras cephalotes														11	14	11	22	34

i) continued.

	Site 1			**Site 2**					**Site 3**					**Site 4**				
	1	**2**	**3**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Diura bicaudata				2	3		1	4	1			1	4					
Isoperla grammatica				2	8	4	1	3		3	4	10	9	1		3	5	
Perlodidae																		
Silo pallipes																		2
Silo sp. (early instar)									3		1					1	1	
Agapetus fuscipes				1	3		1	4		2	7			18	13	226	127	72
Chaetopteryx villosa							1	1				1						
Drusus annulatus	1				8	11	5	35	4	4	19	17	2	1		6	3	7
Potamophylax sp								1				1						
Plectrocnemia conspersa					1	1							1					
Lype phaeopa																		
Tinoides dives					11	3	2	3	2						1		1	
Rhyacophila dorsalis																2		1
Rhyacophila (pupa)					1			1									1	
Rhyacophila obliterata																		
Rhyacophilia septentrionis																		
Dytiscidae (l.)		1																
Oreodytes sanmarkii																		
Elmis aenea (l.)				3	18	80	19	80	14	11	11	3	1	12	26	64	129	80
Elmis aenea (a.)					2	4		4	8	1	3	1	1	25	3	12	4	14
Limnius volckmari (l.)						1												
Limnius volckmari (a.)																1		1
Oulimnius sp. (l.)																		
Oulimnius sp. (a.)																		
Riolus subviolaceus (a.)								3	2					1		3	5	6
Riolus sp. (l.)				4	18	11	13	44	13	4	7	5		17	20	70	17	10
Hydraena sp.						1				1						1		
OLIGOCHAETA	25	16	10	20	1	19	15	9	5	4	5	2		25	8	35	10	11
Ceratopogoniidae	3	12		3	2	5	1	1		1	1				3	5	6	

i) continued

	Site 1			**Site 2**					**Site 3**					**Site 4**				
	1	**2**	**3**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
CHIRONOMIDAE	75	1064	480	30	125	28	24	16	200	136	280	264	13	17	2	89	60	6
Dixa sp.																	2	
Empididae		1		2	3	3						1		1		3		
Dicranota sp.									1							1		
Muscidae													1					
Psychodidae										1	1	2						
Simuliidae																		
Stratiomyidae					1			1										1
Tipulidae																		
HYDRACARINA				1		1	1	2										

ii)

	Site 5					**Site 6**					**Site 7**					**Site 8**			**Site 9**				
	1	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**1**	**2**	**3**	**4**	**5**
Crenobia alpina	1		2	1	1														56	4	38	12	8
Dendrocoelum lacteum																							
Polycelis felina			2			2					1					2			36	80	50	112	40
Piscicola geometra																				1	1		
Ancylus fluviatilis					1					1	4				1				16		11	1	1
Lymnaea peregra					1		3	8	3	4	3	5		1	13	11	22	8					
Lymnaea truncatula																		6					
Sphaeriidae											1				3								6
Zonitoides					1		1																
Asellus aquaticus																							
Asellus meridianus																			1				2
Gammarus pulex	242	41	84	266	400	160	400	624	504	550	88	104	174	268	212	65	92	800	80	84	218	138	208
Baetis sp.	10	9	14	6	12	88	30	72	17	39	48	16	9	120	33	76	46	3	3	2	9	1	
Serratella ignita			1	2	11			4	2	1	1	2				2	6	20			5	1	
Ecdyonurus sp.				1									1			2							
Chloroperla torrentium															1								
Leuctra fusca																22	30	3	5				2
Leuctra hippopus	1		1	1		2						2	12										
Nemoura avicularis																	2						
Nemoura cambrica																			4	1			
Nemurella picteti																							
Protonemoura meyeri	3		12	1	6	4	7	6	28	26		1											
Nemouridae (early instar)													6	1									
Dinocras cephalotes	32	7	15	19	42	2		2	3	1													
Diura bicaudata																							

ii) continued

	Site 5					**Site 6**					**Site 7**					**Site 8**			**Site 9**				
	1	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**1**	**2**	**3**	**4**	**5**
Isoperla grammatica	9	4	3		2		1	1	7	8	1	2		2	2	5	2	4					
Perlodidae											7												
Silo pallipes																							
Silo sp. (early instar)																			1	7			1
Agapetus fuscipes	4			7	2		1	4	4		1	2		2	5				14	90	14	1	112
Chaetopteryx villosa																				1			
Drusus annulatus	1	2		5	6	2	7	6	4	11	20	28		75	34			1	2	7		3	14
Potamophylax sp																				1			6
Plectrocnemia conspersa																1		3			1	1	
Lype phaeopa			1																				
Tinoides dives		3		1	3	1			2	2	2					3							
Rhyacophila dorsalis			1							1													
Rhyacophila (pupa)																							
Rhyacophila obliterata											1												
Rhyacophilia septentrionis																		1		1			
Dytiscidae (l.)																							
Oreodytes sanmarkii																							2
Elmis aenea (l.)	17	17	44	42	206	14	17	26	60	12	6	2	2	2	2	11	8	304	9	5	33	9	
Elmis aenea (a.)	6	2	10	3	3	1		8	4	2	1	1		4				9		2	3	4	
Limnius volckmari (l.)				1																			
Limnius volckmari (a.)	1		1										1										
Oulimnius sp. (l.)													1										
Oulimnius sp. (a.)																1					1		
Riolus subviolaceus (a.)	2		1		11	1	3	4	4	2	2	1			1	1	1	3		3			1
Riolus sp. (l.)	13	8	9	6	63	56	76	360	40	160	5	3	1	1	1		1	3	14	13	16	19	
Hydraena sp.	1				1	1		3		1					3	1	4	7		1	2	1	1
OLIGOCHAETA	6	1	2	7	3	1		1	1		10	10	8	8	6	15	44	1	2		1		9
Ceratopogoniidae	1		1	1	1						18	2	8	5	7		1	2			1	2	6

ii) continued

	Site 5					Site 6					Site 7					Site 8			Site 9				
	1	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**1**	**2**	**3**	**4**	**5**
CHIRONOMIDAE	48	3	190	24	150	25	145	100	195	450	12	9	4	29	12	80	130	760	360	150	1280	80	140
Dixa sp.																							
Empididae	1			1	2	2		2		1		3				1		5					
Dicranota sp.								1			1	1	1	2	4				1		1		
Muscidae																	4			1			
Psychodidae																1							1
Simuliidae						1	1		3	4													
Stratiomyidae					1										1			1					2
Tipulidae																1							
HYDRACARINA					1												1	2		1		2	5

APPENDIX 2 Raw baseline survey data for the benthic macroinvertebrate community of the River Glen: i) sites 1-4; ii) sites 5-8. Site locations provided in Figure 3.8.

i)

	Site 1			**Site 2**			**Site 3**					**Site 4**			
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**
Dendrocoelom lacteum														1	1
Dugesia lugubris OR *polychroa*						26								2	1
Polycelis nigra OR *tenuis*				34	7							4	6	5	9
Erpobdella octoculata	8	3	5	23	39	36	27	12	27	29	54	24	48	40	14
Glossiphonia complanata	2	1	3	5	1	7	17	2	1	3	64	11	21	7	6
Helobdella stagnalis				2	2	1		1	1	1		2	1	2	
Theromyzon tessulatum	1		1						1						
Piscicola geometra							4	1	2	4	6	3	3	5	7
Ancylus fluviatilis							51	36	15	23	11			1	
Bithynia tentaculata															
Lymnaea peregra			1												
Lymnaea spp.															
Physa fontinalis								1				4	3	4	
Planorbidae			1												
Planorbis albus															
Planorbis corneus	1														
Planorbis contortus												1		2	2
Planorbis vortex							1	1	2	1	4	3	2	7	1
Potamopyrgus antipodarum							122	15	22	15	1				
SPHAERIIDAE	26	1	15	49	4	40	214	23	126	93	480	17	48	16	3
Succinea spp.															
Valvata cristata	1						1			1	1	1			1
Valvata piscinalis															

i) continued

	Site 1			**Site 2**			**Site 3**					**Site 4**			
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**
ZONOITIDAE											1				
Asellus aquaticus				84	39	88	2	2	1	5	2	41	34	61	45
Asellus meridianus	7					2								4	
Crangonyx pseudogracilis												4	5	3	2
Gammarus pulex	110	152	124	23	13	44	146	59	83	92	184	9	16	3	21
Niphargus aquilex						1									
COPEPODA															
OSTRACODA				11	1	3			1				3		
Baetis spp.	2	19	18	1	1	1	61	93	62	57	42	25	2	10	17
Procloeon bifidum															
Caenis horaria															
Caenis luctuosa	15	1	1	1								105	120	32	39
Ephemera danica												3	3	3	2
Serratella ignita							2	5	3	1	5	5	8	4	6
Habrophlebia fusca	1		2												
TRICHOPTERA (early instar)	1						1		1	1					
Agapetus fuscipes	1	1	1				10	4	7	14	6				
Goera pilosa (larvae)	5	5	4									3	1	12	3
Goera pilosa (pupae)															
Hydropsyche angustipennis	4	8	37									4	27	13	2
Hydropsyche pellicidula												1			
Hydropsyche siltalai							1			1	1				1
Hydroptila spp. (larvae)							2		1	4		2			
Hydroptila spp. (pupae)							1				1				
Lepidostoma hirtum									1		1			16	4
Athripsodes binelineatus							1					2	11	22	3
Mystacides azurea													3		
Mystacides longicornis group												2			
Limnephilus extracticus			1			1									

i) continued

	Site 1			Site 2			Site 3					Site 4			
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**
Limnephilidae spp.	2	1											1		
Molanna angustata															
Plectrocnemia conspersa														1	
Polycentropus flavomaculatus												1	1		3
Psychomyidae (early instar)															
Lype reducta													1		
Tinoides waeneri							12	3	2	1			1		
Tinoides sp.	1														
Rhyacophilidae sp. (early instar)															
Rhyacophila dorsalis										1					
Sericostoma personatum (larvae)							14		2		3	25	12	2	
Sericostoma personatum (pupae)								1				1			
Potamonectes depressus elegans												1	1		
Elmis aenea (larvae)	15	9	19	5	1	7	2		1	2		31	132	180	31
Elmis aenea (adult)	4		6			3						4	8	2	3
Limnius volckmari (larvae)															
Oulimnius spp. (larvae)	80	10	19	4	3	7	8	3	1		8	72	141	126	50
Oulimnius spp. (adult)	11	5	5		1	1		1				8	23	9	9
Riolus subviolaceus (larvae)															
Gyrinidae (larvae)															
Haliplus lineatocollis				3											
Haliplidae (larvae)	1			1	1		1	1					1		2
Helophorus brevipalpis					2	2									
Hydraena spp. (adult)			1												
OLIGOCHAETA	81	9	38	268	93	120	108	64	79	108	44	80	168	159	158
CERATOPOGONIDAE	5	4	2	1	1	3	1	1	4	4		7	3	23	8
CHIRONOMIDAE	64	29	22		1	3	235	161	175	182	136	28	63	23	29
Diptera (other)										1					
EMPIDIDAE							9	4	4	12	5				

i) continued

	Site 1			**Site 2**			**Site 3**					**Site 4**			
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**
MUSCIDAE	1	6	1				3	2		4		1		1	
Dicranota spp.	2		3				3		3	4	3			2	
PSYCHODIDAE				3	6	8	3	1	1	2		1			
SIMULIIDAE (larvae)		3	1		1		3	37	34	35	570	2	5	9	44
SIMULIIDAE (pupae)								1	6	3	67				
TABANIDAE					1										
TIPULIDAE	5		2		9	16			2	2		7	2	9	1
HYDRACARINA		1					3	2			1	4	9	6	1
Sialis lutaria															

ii)

	Site 5			Site 6				Site 7				Site 8				
	1	**2**	**3**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**5**
Dendrocoelom lacteum	1		3											3		
Dugesia lugubris OR *polychroa*								5	17	2	9	1		2		
Polycelis nigra OR *tenuis*		1						8	13	14	12	15	1	28	30	4
Erpobdella octoculata	8	35	25	1	3	7	3	6	24	28	17	4		9	8	2
Glossiphonia complanata		8	8	4	1	1	2	3	6	3	4	1	1	4	6	1
Helobdella stagnalis			7			1		2	3	1	8	4		2	1	1
Theromyzon tessulatum																
Piscicola geometra		2	4													
Ancylus fluviatilis	77	15	48													
Bithynia tentaculata		10	12		3	5	3	21	35	21	42		1	1	7	2
Lymnaea peregra																
Lymnaea spp.										1	1				1	
Physa fontinalis			1		1	3		1						1	1	
Planorbidae																
Planorbis albus			1													
Planorbis corneus						1	1					2			1	
Planorbis contortus		3	4									2		1	3	
Planorbis vortex	1	24	22					5	3	1					2	
Potamopyrgus antipodarum	15	32	51		6	1	3									
SPHAERIIDAE	2	22	76		2	1	1		2	2	9	72	29	74	140	32
Succinea spp.	1	1	1		3		3									
Valvata cristata	1	1	1					1	7	6	24				1	
Valvata piscinalis	3	13	8				1					1	1		4	1
ZONOITIDAE																
Asellus aquaticus	8	21	22	2	3		2	70	51	68	52	101	63	61		50
Asellus meridianus								14	76	52	38	3				
Crangonyx pseudogracilis		2							2		3		4	68	80	
Gammarus pulex	61	158	132	42	48	47	44	12	21	20	12	375	88	100	308	146

ii) continued

	Site 5			Site 6				Site 7				Site 8				
	1	**2**	**3**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**5**
Niphargus aquilex				1												
COPEPODA																
OSTRACODA													1		2	
Baetis spp.	18	6	10	3	4	1	2	68	92	38	97	33	10	6	12	9
Procloeon bifidum					1											
Caenis horaria					1			1			2					
Caenis luctuosa	4	24	10	1	11	16	25	6	23	43	95	5			3	1
Ephemera danica		1	3									10	1	6	3	
Serratella ignita															1	
Habrophlebia fusca				7	8	3	4									
TRICHOPTERA (early instar)						1								2		
Agapetus fuscipes	2	2	1	31	18	32	5	10	13	1						
Goera pilosa (larvae)	1	11	3	1	2	11	7							1		
Goera pilosa (pupae)															1	
Hydropsyche angustipennis	19	10	21	1	36	9	8						1	1		
Hydropsyche pellicidula														1		
Hydropsyche siltalai		3														1
Hydroptila spp. (larvae)					1			1						2		
Hydroptila spp. (pupae)												3		1		
Lepidostoma hirtum	6	120	53												1	
Athripsodes binelineatus	2	19	6					5	10	16	12	3	3	2	2	2
Mystacides azurea																
Mystacides longicornis group		1														1
Limnephilus extracticus																
Limnephilidae spp.											1				1	
Molanna angustata		1	1													
Plectrocnemia conspersa							2									
Polycentropus flavomaculatus		1										30	43	46	45	10
Psychomyidae (early instar)		1														

ii) continued

	Site 5			**Site 6**				**Site 7**				**Site 8**				
	1	**2**	**3**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**5**
Lype reducta																
Tinoides waeneri		2			1		6					2	1			1
Tinoides sp.																
Rhyacophilidae sp. (early instar)												1				
Rhyacophila dorsalis																
Sericostoma personatum (larvae)			1					1				11		3	19	7
Sericostoma personatum (pupae)																
Potamonectes depressus elegans																
Elmis aenea (larvae)	42	130	52	1	27	7	16	1	2	1	2	37	29	50	31	20
Elmis aenea (adult)	2	6	6		2								2	6	2	1
Limnius volckmari (larvae)												2		5	2	3
Oulimnius spp. (larvae)	10	43	41	3	16	8	26	25	94	135	114	60	7	16	14	14
Oulimnius spp. (adult)	2	5	3	1	3	1	1		1	1	2			4		2
Riolus subviolaceus (larvae)								1								
Gyrinidae (larvae)														1		
Haliplus lineatocollis																
Haliplidae (larvae)					1	1		6	7	14	16	1		2		2
Helophorus brevipalpis																
Hydraena spp. (adult)																
OLIGOCHAETA	235	163	148	132	68	19	53	51	102	52	36	210	50	148	260	10
CERATOPOGONIDAE	8		3	2	4		3				1	22	1	6	8	3
CHIRONOMIDAE	336	180	29	4	50	30	76	6	10	6	9	740	228	530	592	53
Diptera (other)																
EMPIDIDAE	1		5		1	1	2									1
MUSCIDAE		1		2	1			5	9	8	12	2	1		1	
Dicranota spp.		7			2		1	1	1							
PSYCHODIDAE			1													
SIMULIIDAE (larvae)	768	143	5						4	1	5	18		10	30	19
SIMULIIDAE (pupae)									1							

ii) continued

	Site 5			**Site 6**				**Site 7**				**Site 8**				
	1	**2**	**3**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**5**
TABANIDAE																
TIPULIDAE			4					11	18	18	9	1				
HYDRACARINA	4	5			3		3		1	1	1					
Sialis lutaria												22	7	24	23	

APPENDIX 3 Raw baseline survey data for the hyporheic invertebrate community of River Lathkill sites 1-5. Site locations provided in Figure 3.11.

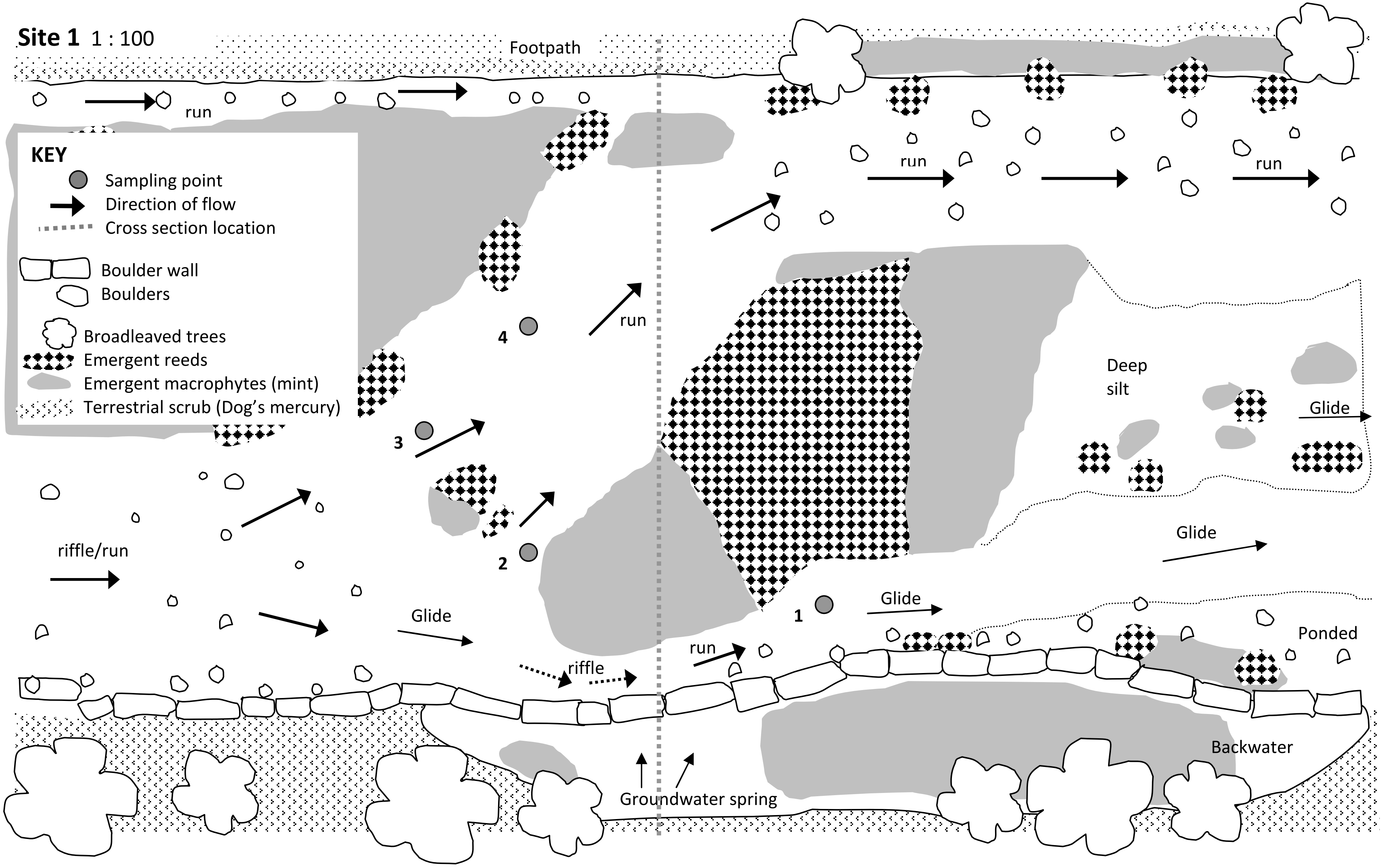
	Site 1					**Site 2**					**Site 3**					**Site 4**					**Site 5**				
	1	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Dugesia lugubris group															1										
Polycelis felina	51	19		4	22					1		1													
Polycelis nigra OR *tenuis*													1									1			2
Lymnaea peregra																						2			
Asellus meridianus		1									1														
Gammarus pulex	2			2	7		3			1	9	2	1	2	1	2			2	1	3	6	5	7	
CYCLOPOIDA	2	5		4											2			6				1			1
OSTRACODA	1						1				1								1		5	10	4		
Baetis spp.									1		1		2			2	3					1		2	
Serratella ignita				2							1		1						1		1	8	7	4	
Isoperla grammatica													1								1				1
Leuctra spp.																1		2				1			
Nemoura cambrica													3												
Nemoura spp.		1																							
Agapetus fuscipes					1		7	17	5	8		2			1		4		1						
Drusus annulatus	2																								
Micropterna spp																									1
Elmis aenea (larvae)	1						1				1		2	1											
Oulimnius spp. (larvae)				1									1					1							
NEMATODA			1		1	1	4		2			2	1		3	1			2	3					
OLIGOCHAETA	5		1	2	1	4	1	1	3		1			3	1		3			2					
Ceratopogonidae																		1							
Chironomidae (larvae)	2	1		6	1			2		1	9	2	6		1	3	2	6			1		2	3	1
Simuliidae (larvae)													1												
HYDRACARINA							1															1			

APPENDIX 4 Raw baseline survey data for the hyporheic invertebrate community of River Glen sites 1-4. Site locations provided in Figure 3.12.

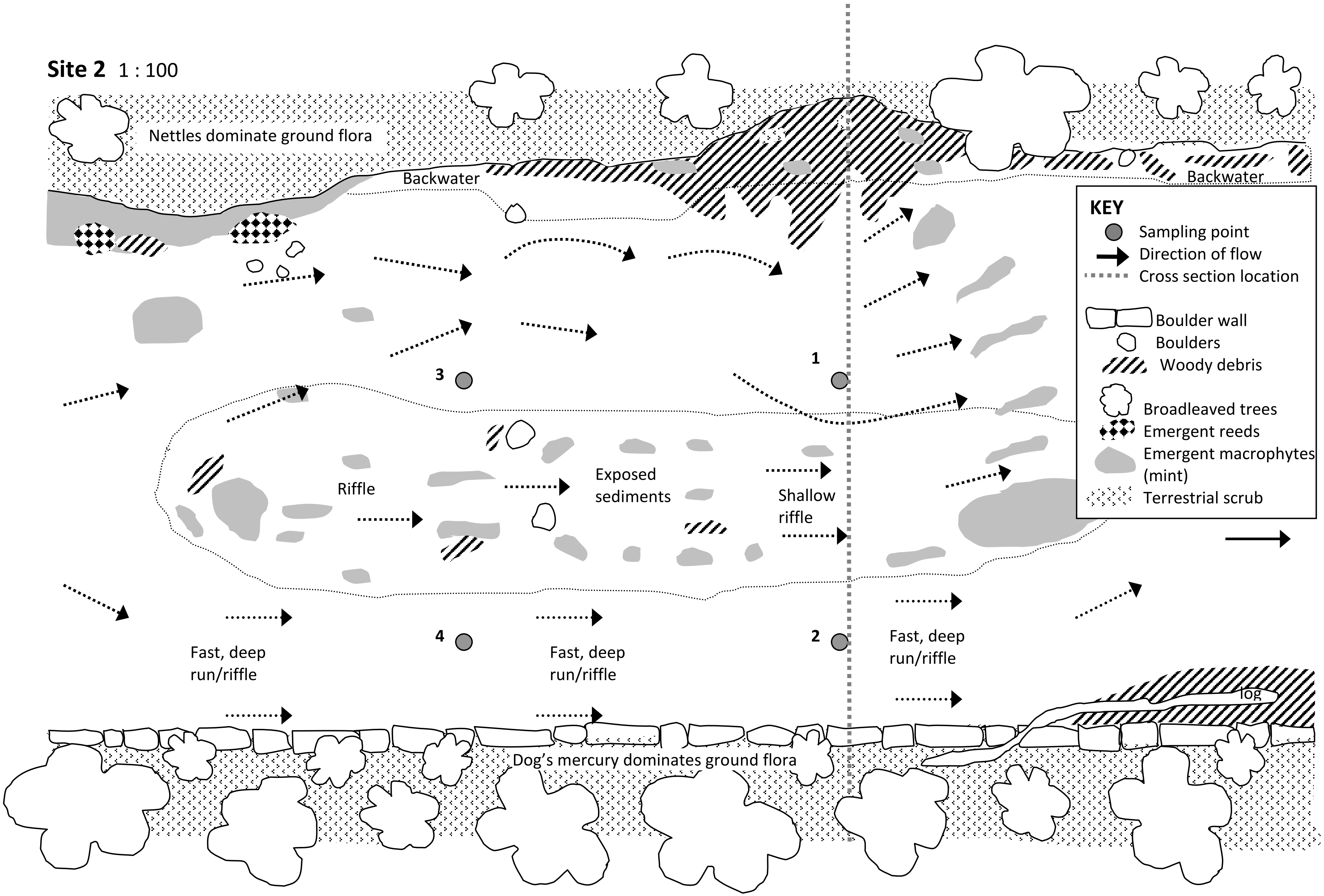
	Site 1					**Site 2**				**Site 3**				**Site 4**				
	1	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**5**
Polycelis nigra OR *tenuis*																	1	
Potamopyrgus antipodarum				1									1					
Sphaeriidae											1							
Valvata cristata						1											2	
Gammarus pulex											8							
CYCLOPOIDA										1								
Baetis spp.	1																	
Caenis luctuosa group						1												1
Limnephilidae (early instar)												1						
Elmis aenea (adult)								1										
Oulimnius spp. (larvae)								1	2									
NEMATODA		1				1				1	2		1		1	3		
OLIGOCHAETA	1			1		6		1	3	4	2		5	2	3	6	16	3
Ceratopogonidae						1		1	1									
Chironomidae	1	7	1	1	2	22		18	7	4	23	8	12	3	2	2	11	13
DIPTERA (pupae)																	1	
Simuliidae						1									1		1	
HYDRACARINA						1				1								

APPENDIX 5 Plan views of River Lathkill study sites

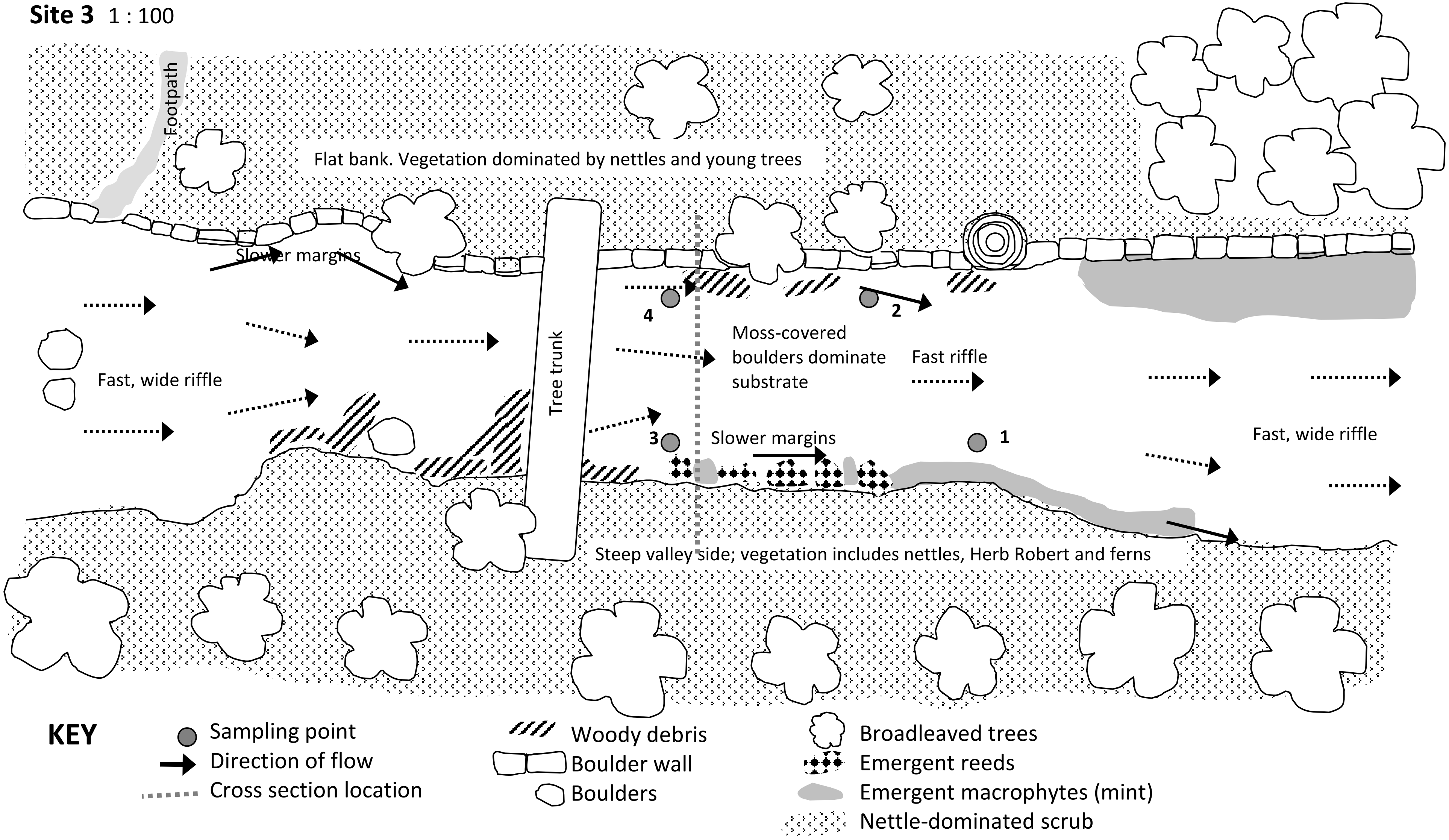
Site 1 1 : 100


Footpath
run
KEY
Sampling point
Direction of flow
Cross section location
Boulder wall
Boulders
Broadleaved trees
Emergent reeds
Emergent macrophytes (mint)
Terrestrial scrub (Dog's mercury)
run
run
run
run
4
3
2
1
Deep
silt
Glide
Glide
Glide
Glide
riffle/run
Glide
riffle
run
Ponded
Backwater
Groundwater spring

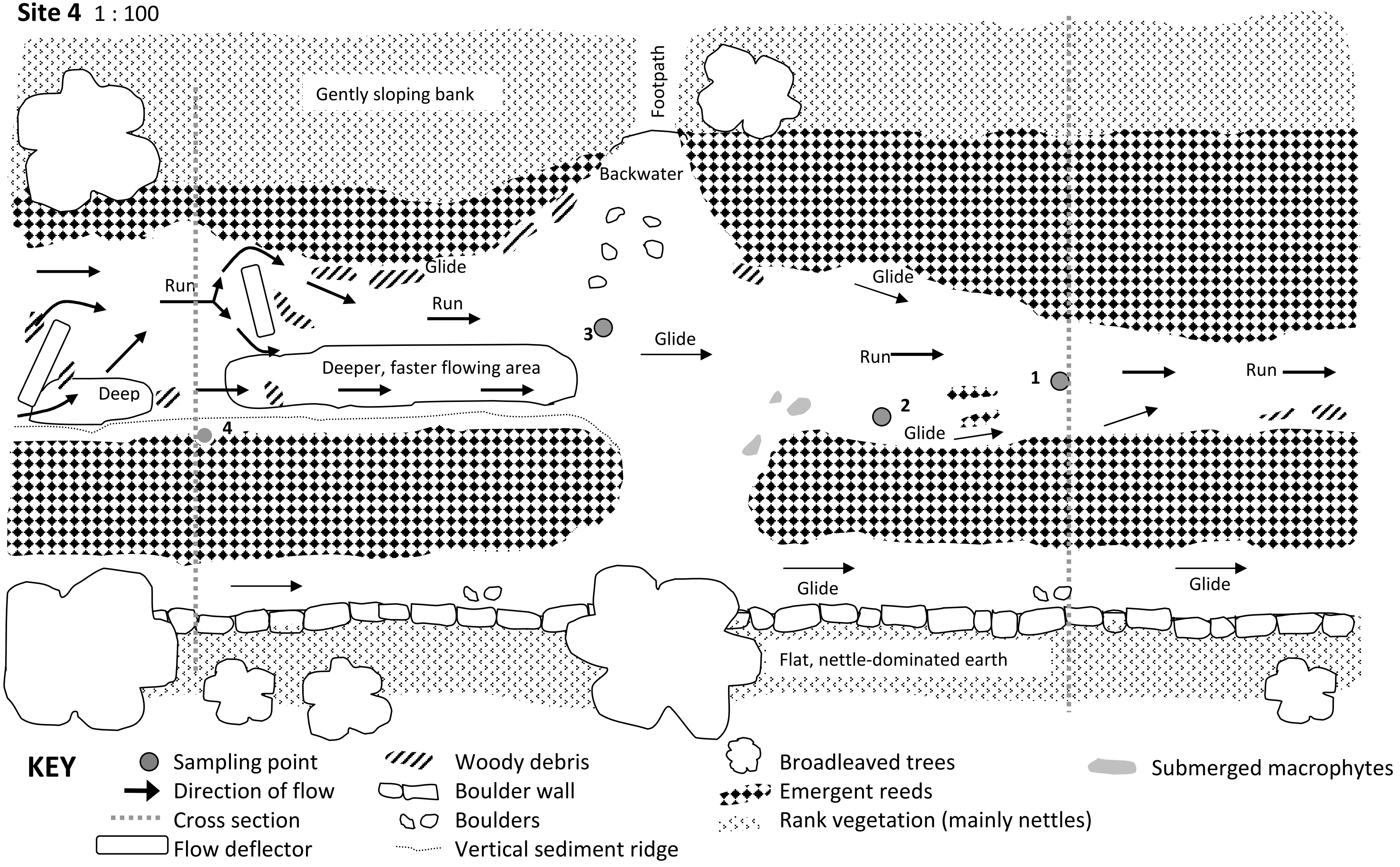
Site 2 1 : 100


Nettles dominate ground flora
Backwater
Backwater
KEY
Sampling point
Direction of flow
Cross section location
Boulder wall
Boulders
Woody debris
Broadleaved trees
Emergent reeds
Emergent macrophytes (mint)
Terrestrial scrub
3
1
Riffle
Exposed sediments
Shallow riffle
4
2
Fast, deep run/riffle
Fast, deep run/riffle
Fast, deep run/riffle
log
Dog's mercury dominates ground flora

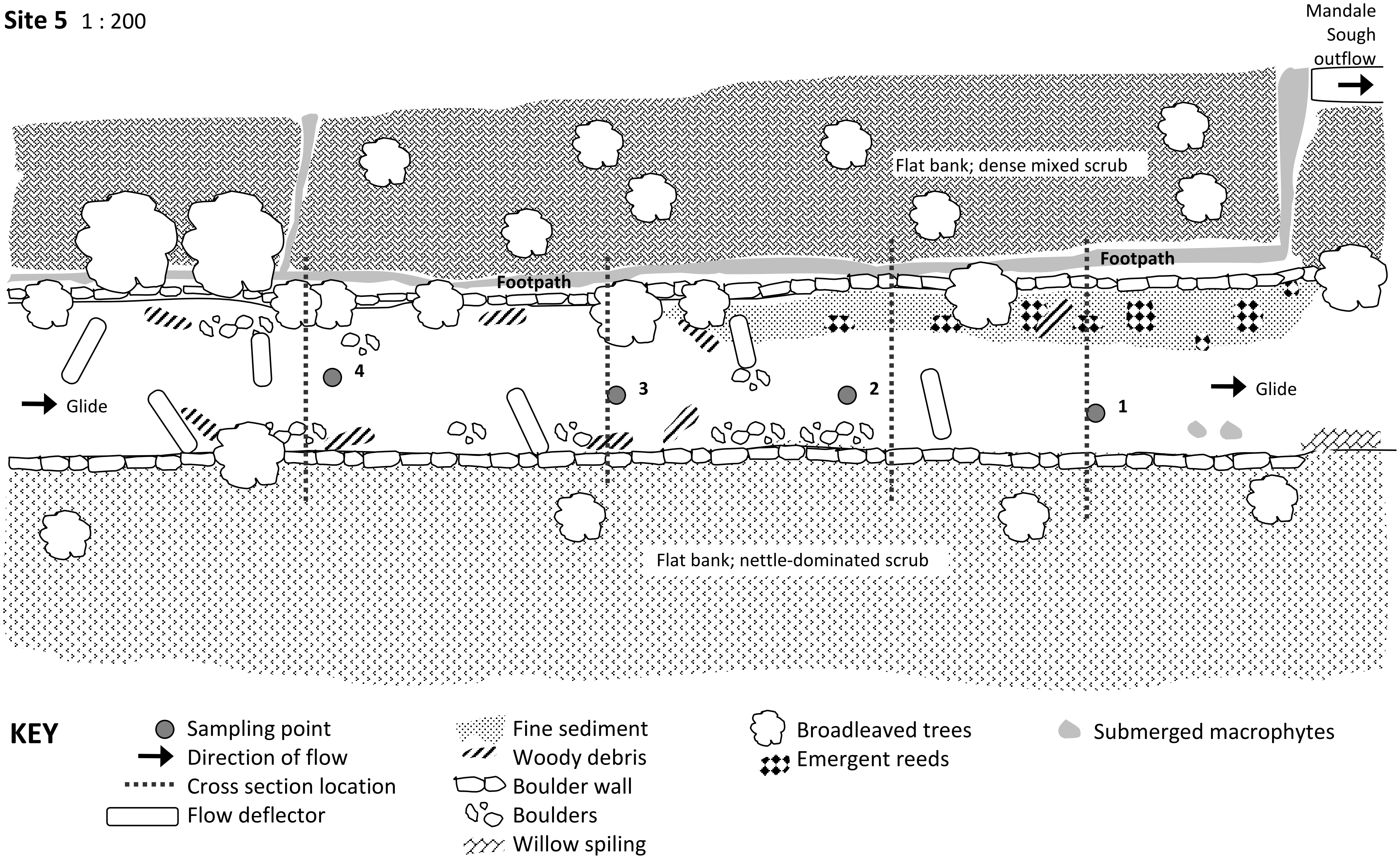
Site 3 1 : 100


Footpath
Flat bank. Vegetation dominated by nettles and young trees
Slower margins
4
2
Moss-covered boulders dominate substrate
Fast riffle
Fast, wide riffle
Tree trunk
3
Slower margins
1
Fast, wide riffle
Steep valley side; vegetation includes nettles, Herb Robert and ferns
KEY
Sampling point
Direction of flow
Cross section location
Woody debris
Boulder wall
Boulders
Broadleaved trees
Emergent reeds
Emergent macrophytes (mint)
Nettle-dominated scrub

Site 4 1 : 100


Gently sloping bank
Footpath
Backwater
Glide
Run
Run
Glide
Glide
Run
3
Glide
Run
Deeper, faster flowing area
Deep
1
Run
2
Glide
4
Glide
Glide
Flat, nettle-dominated earth
KEY
Sampling point
Direction of flow
Cross section
Flow deflector
Woody debris
Boulder wall
Boulders
Vertical sediment ridge
Broadleaved trees
Emergent reeds
Rank vegetation (mainly nettles)
Submerged macrophytes

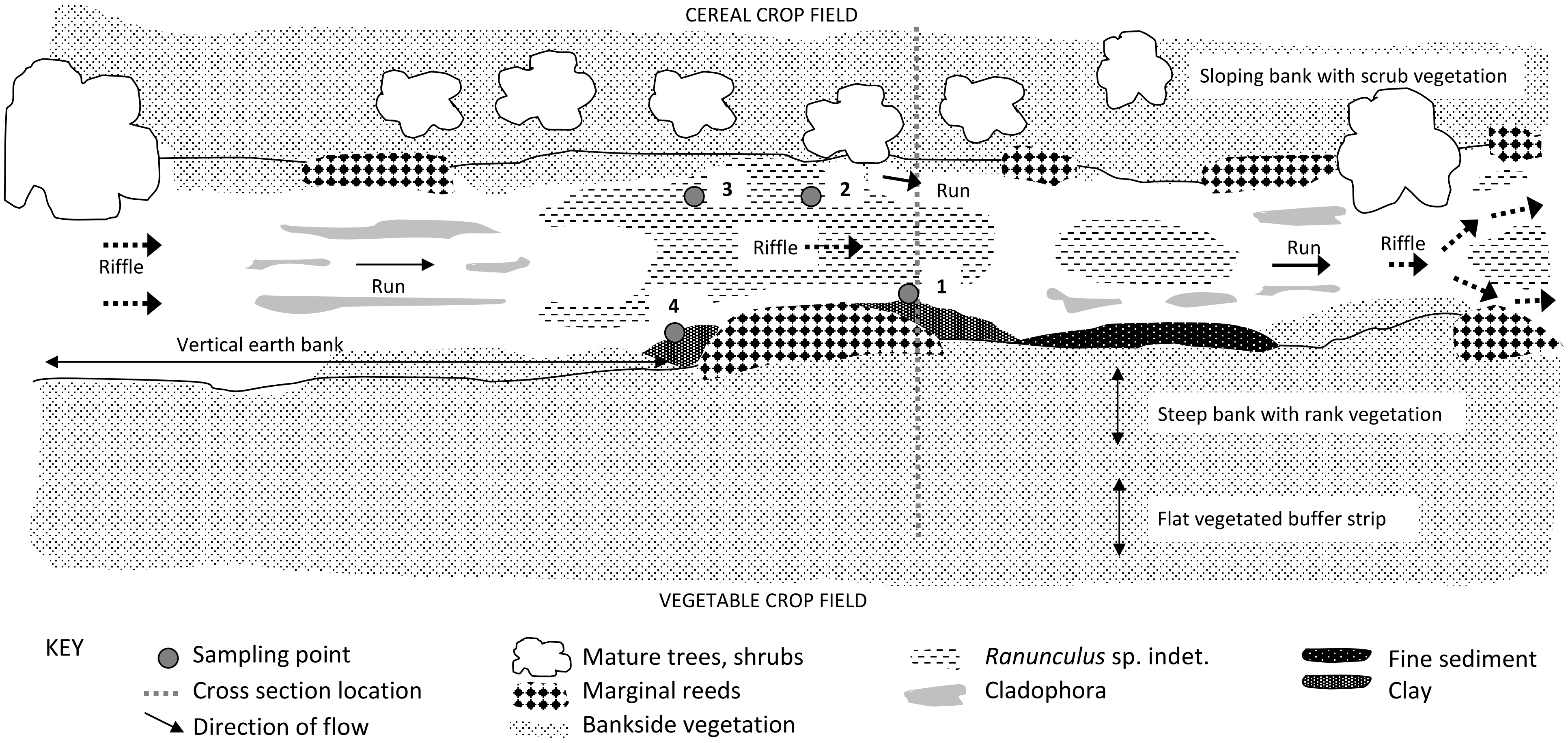
Site 5 1 : 200


Mandale Sough outflow
Flat bank; dense mixed scrub
Footpath
Footpath
4
3
2
1
Glide
Glide
Flat bank; nettle-dominated scrub
KEY
Sampling point
Direction of flow
Cross section location
Flow deflector
Fine sediment
Woody debris
Boulder wall
Boulders
Willow spiling
Broadleaved trees
Emergent reeds
Submerged macrophytes

APPENDIX 6 Plan views of River Glen study sites

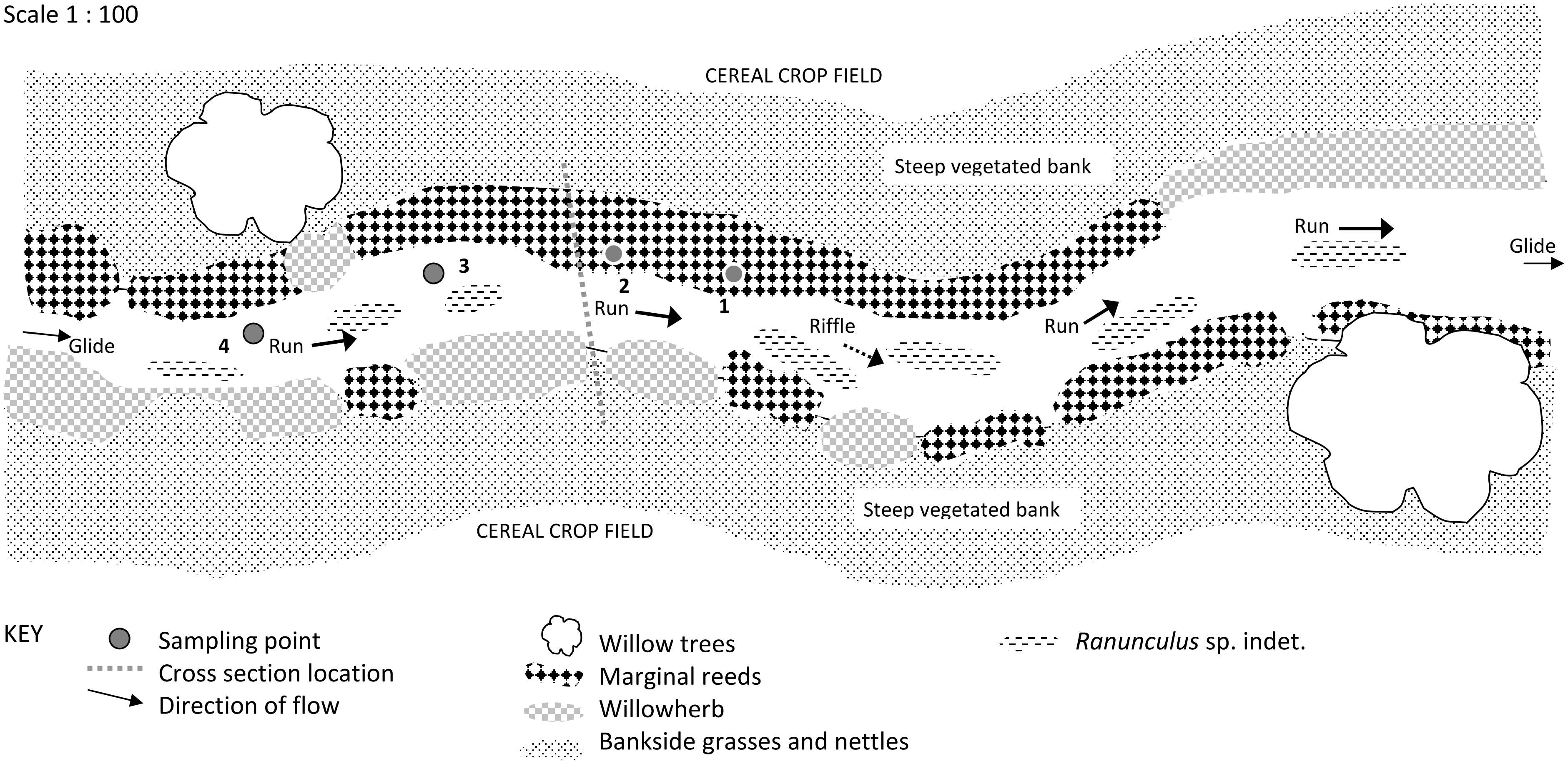
Site 1

Scale 1 : 100



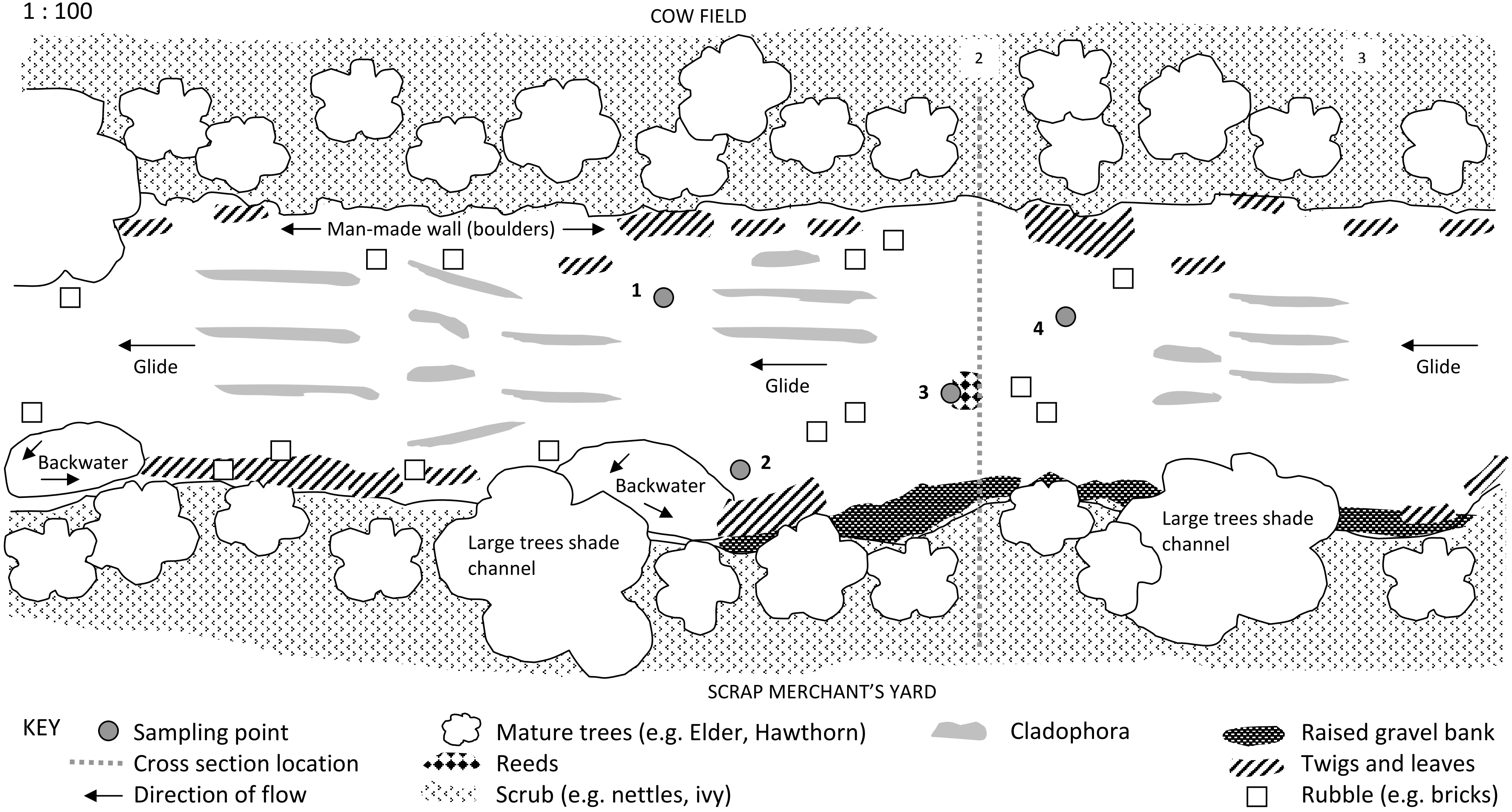
Site 2

Scale 1 : 100



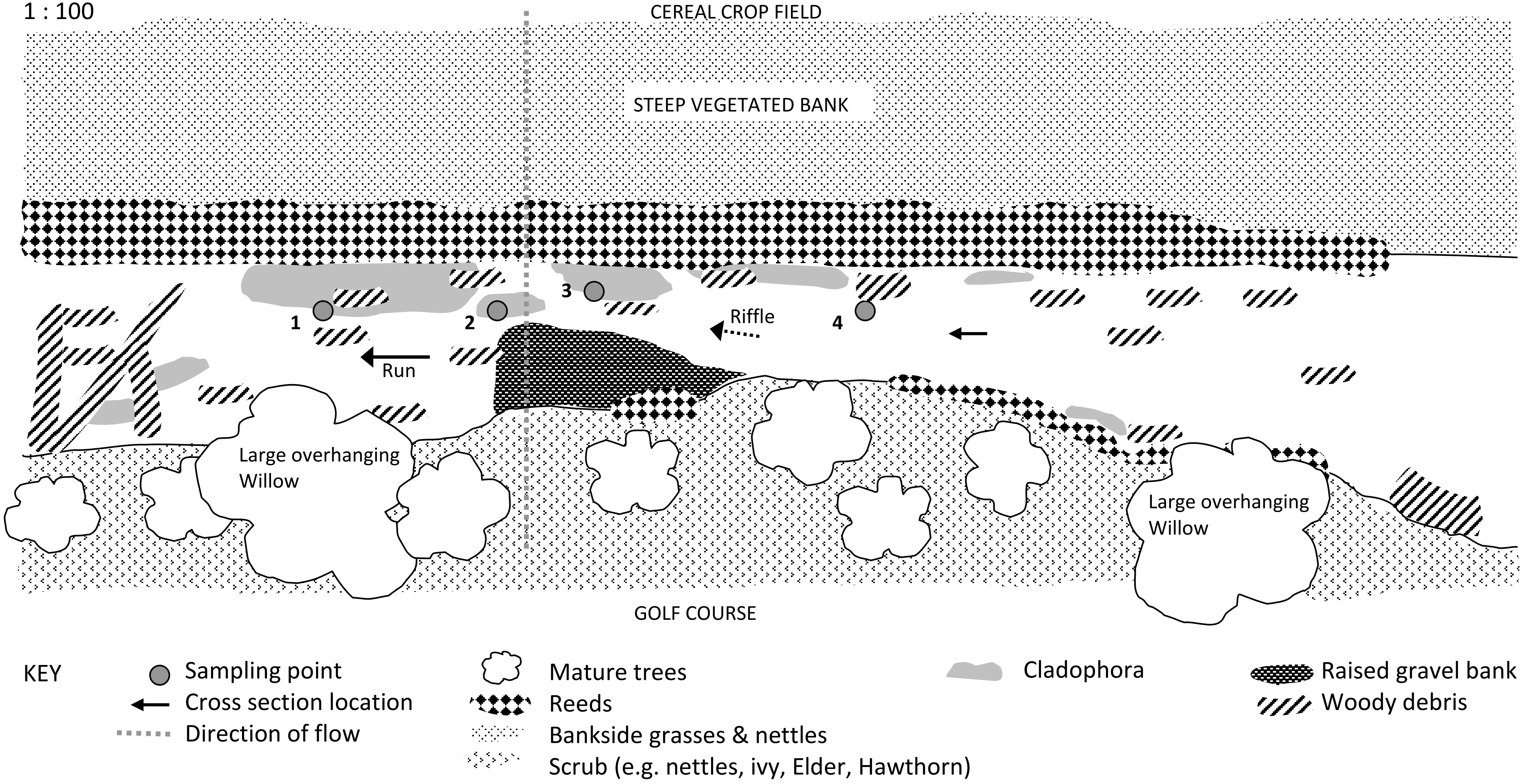
Site 3

1 : 100


COW FIELD
2
3
Man-made wall (boulders)
1
4
Glide
Glide
Glide
3
Backwater
2
Backwater
Large trees shade channel
Large trees shade channel
SCRAP MERCHANT'S YARD
KEY
Sampling point
Cross section location
Direction of flow
Mature trees (e.g. Elder, Hawthorn)
Reeds
Scrub (e.g. nettles, ivy)
Cladophora
Raised gravel bank
Twigs and leaves
Rubble (e.g. bricks)

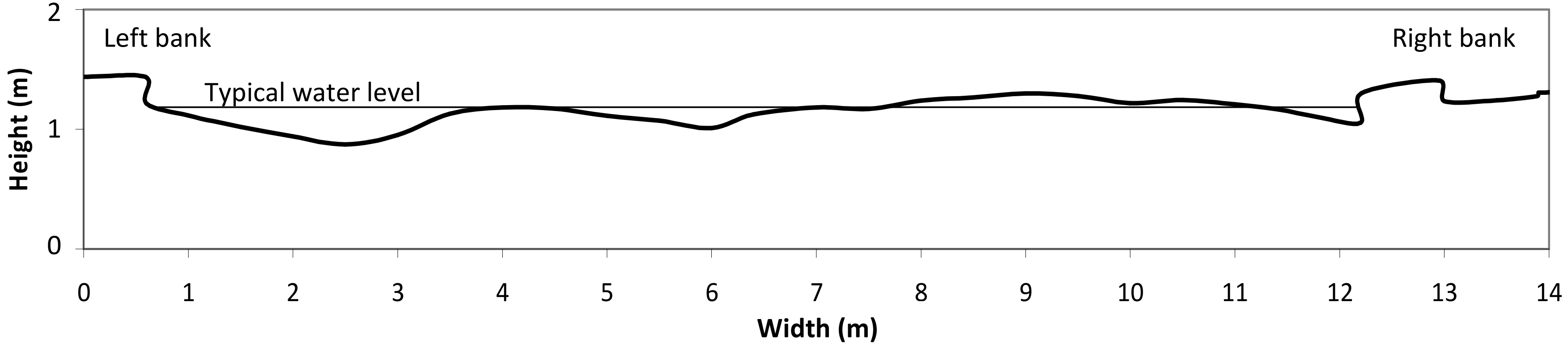
Site 4

1 : 100

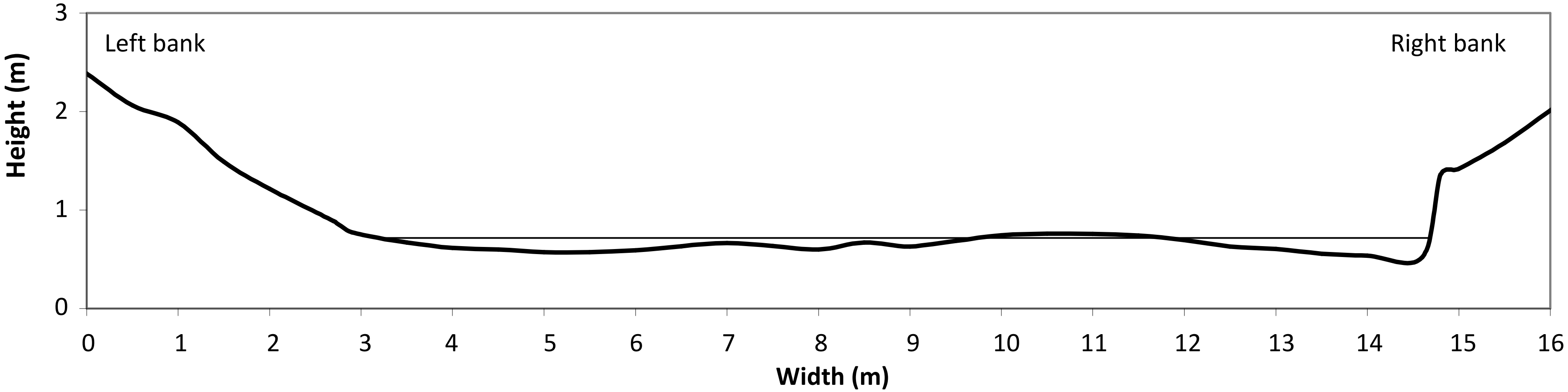

CEREAL CROP FIELD
STEEP VEGETATED BANK
3
1
2
4
Riffle
Run
Large overhanging Willow
Large overhanging Willow
GOLF COURSE
KEY
● Sampling point
← Cross section location
Direction of flow
Mature trees
Reeds
Bankside grasses & nettles
Scrub (e.g. nettles, ivy, Elder, Hawthorn)
Cladophora
Raised gravel bank
Woody debris

APPENDIX 7 Cross-sectional profiles of River Lathkill study areas

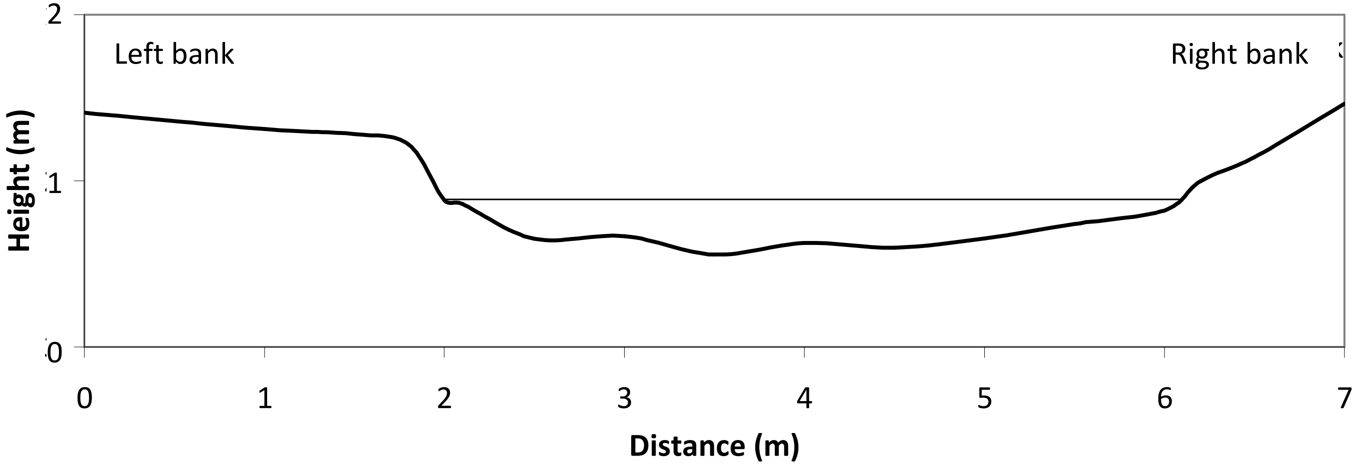
Site 1


Left bank
Right bank
Typical water level
Height (m)
0
1
2
Width (m)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14

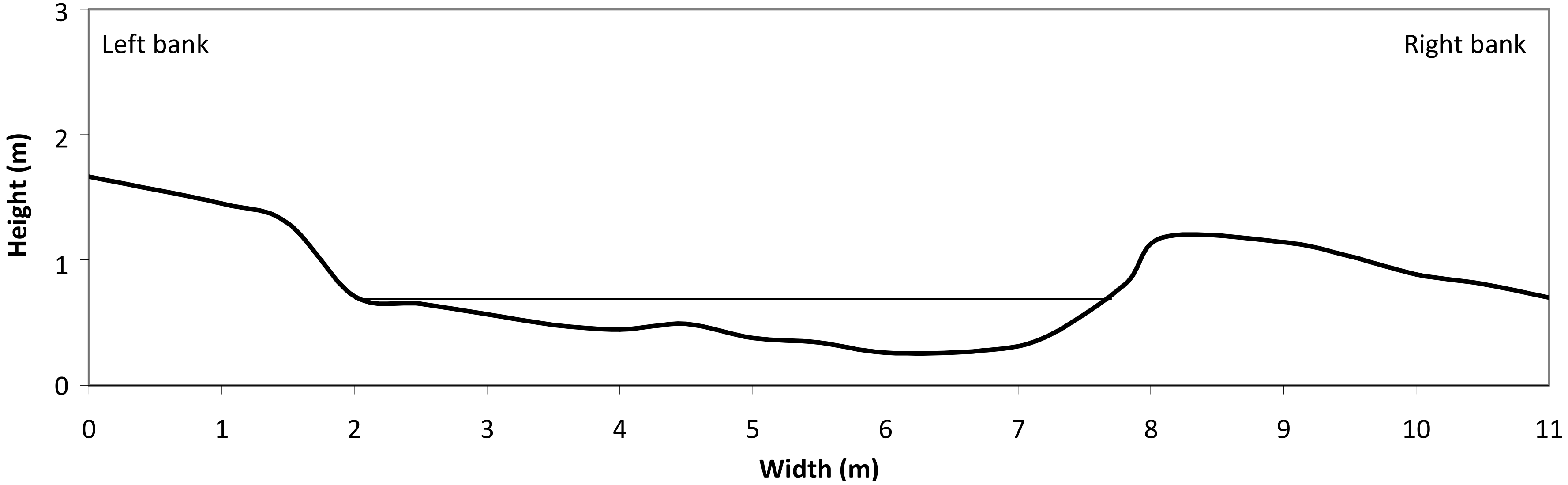
Site 2


Left bank
Right bank
Height (m)
0
1
2
3
Width (m)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

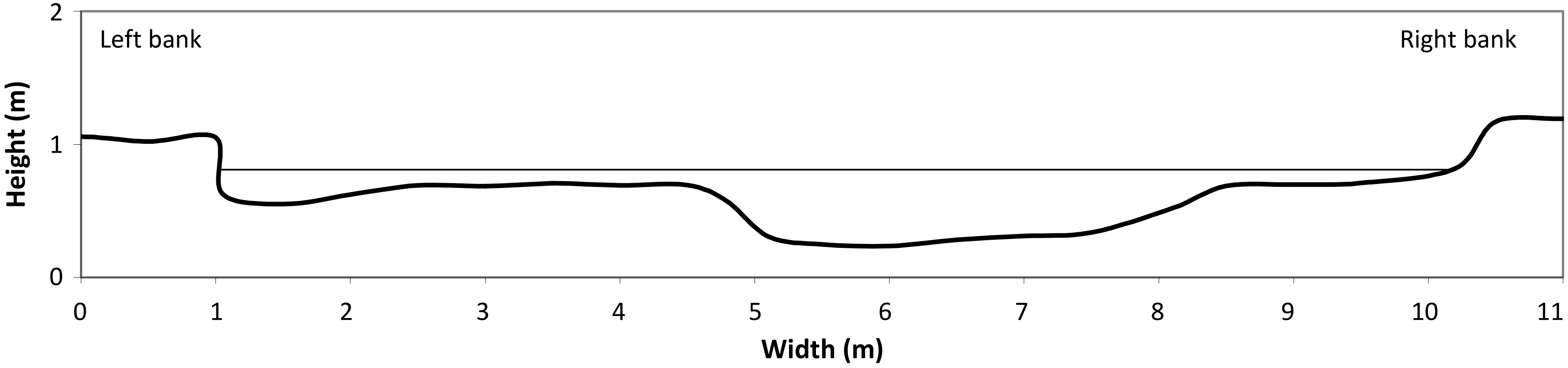
Site 3


Left bank
Right bank
Height (m)
2
1
0
0
1
2
3
4
5
6
7
Distance (m)

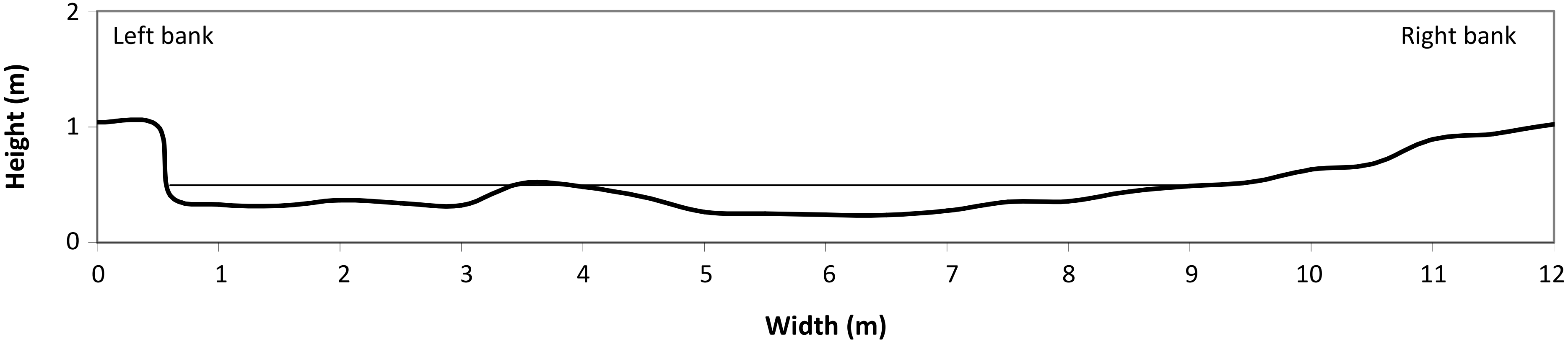
Site 5


Left bank
Right bank
Height (m)
3
2
1
0
0
1
2
3
4
5
6
7
8
9
10
11
Width (m)

Site 4, upstream area

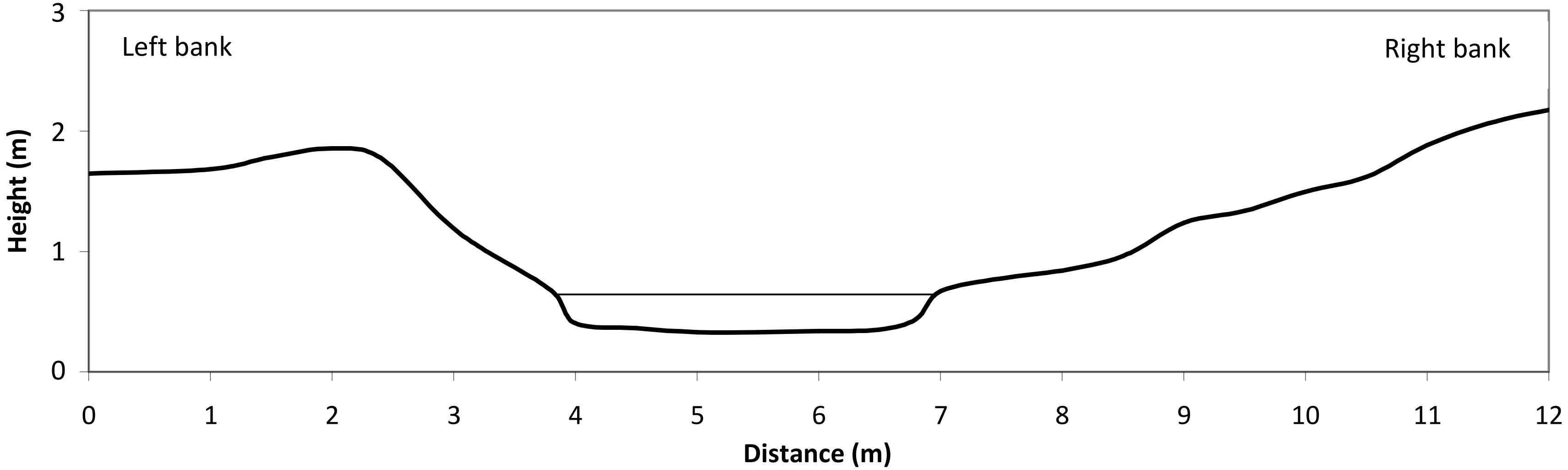


Site 4, downstream area

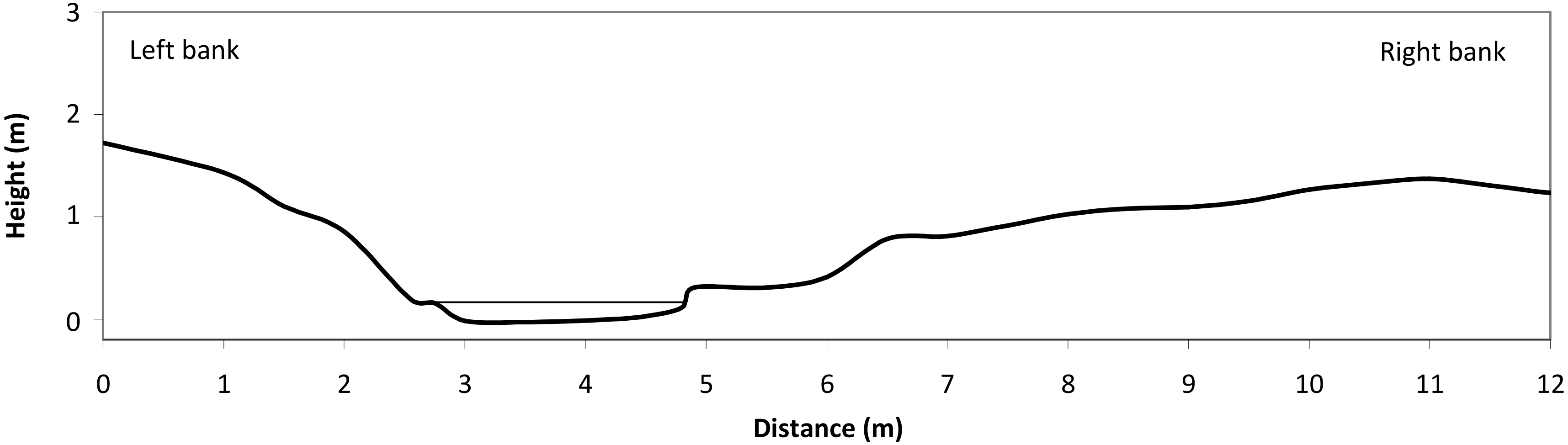


APPENDIX 8 Cross-sectional profiles of River Glen study areas

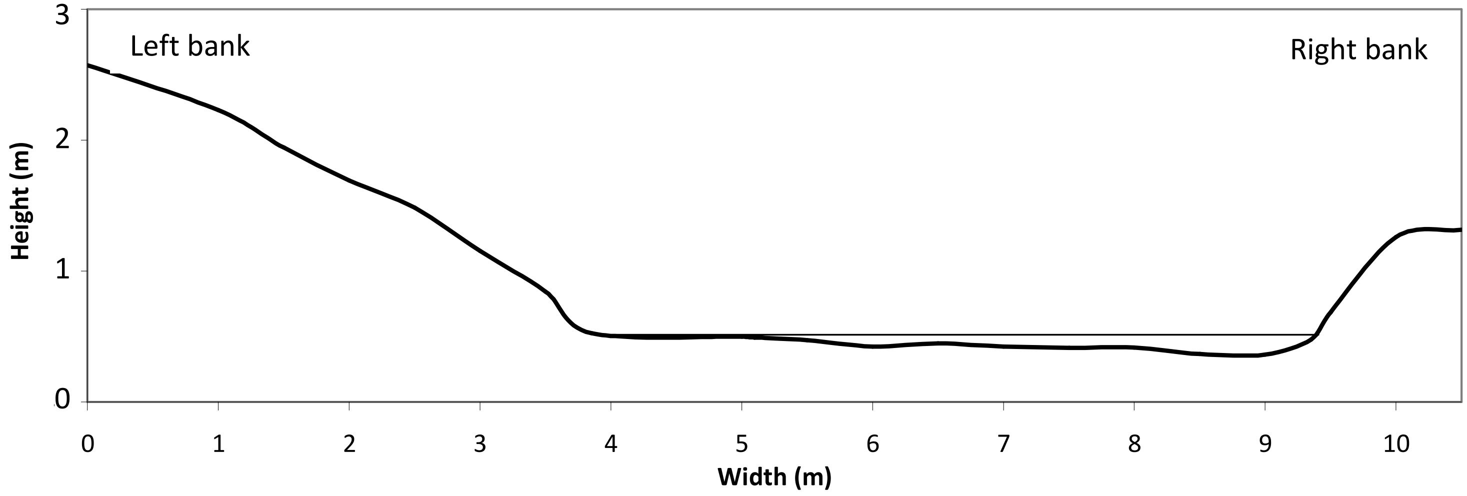
Site 1


Left bank
Right bank
Height (m)
0
1
2
3
Distance (m)
0
1
2
3
4
5
6
7
8
9
10
11
12

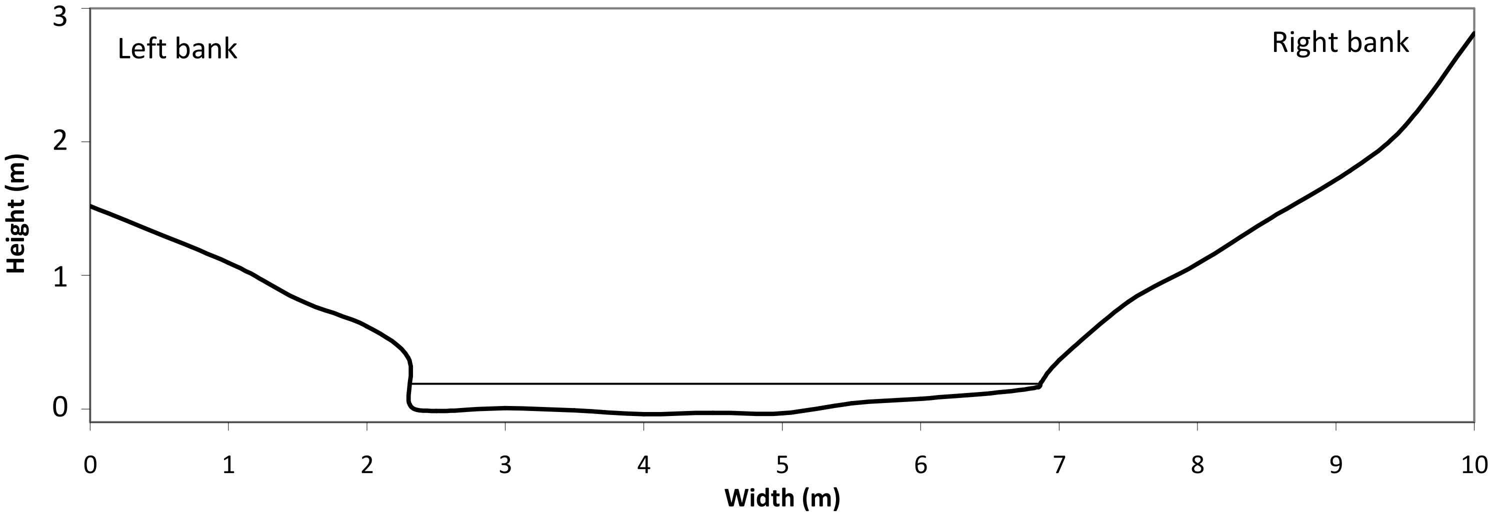
Site 2


Left bank
Right bank
Height (m)
0
1
2
3
Distance (m)
0
1
2
3
4
5
6
7
8
9
10
11
12

Site 3, upstream area



Site 3, downstream



Site 4